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

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

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THE RATIONALE, DESIGN AND METHODS OF NEW STUDIES

ORIGINAL PAPER

JMS Journal of Medical Science

Application of a bottom-up approach to reduce healthcare disparity between the urban and rural areas

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ABSTRACT

Disparity in access to healthcare between rural and urban regions occurs world-wide, both in higher and lower income countries. To offset poor health outcomes a number of approaches to structuring healthcare services can be used. Several factors have been identified to play a role, however there are differing degrees of severity in how they contribute to the healthcare gap - depending on whether a higher or lower income country is being evaluated. Traditionally, healthcare systems worldwide adopt a top-down approach which is initiated by large institutions providing resources required for large scale projects along with centralisation of efforts. Although it does lead to change, the results can be short-lived. The authors discuss the bottom-up approach used in ASHWINI organisation in Gudalur, India which allowed for the development of accessible and sustainable healthcare system managed by the community. Other projects, based in part on the principles of a bottom-up approach, have been applied in other countries to reduce healthcare disparities. When designed to deliver geographically-accessible, locally managed, culturally appropriate care, the bottom-up approach can provide sustainable results and being universal in nature, it could be applied in other setting with similar set up.

Introduction

A world-wide disparity is observed in access to healthcare between the rural and urban regions. Literature suggests that the rural areas tend to have poorer health outcomes compared to their urban counterparts, with physical, cultural and resource-related factors which affect the inequality in the provision of healthcare. The disparity is observed in countries with high and low Gross National Income (GNI) per capita alike, although a greater difference is visible in the states with a lower GNI per capita.

Improving access to healthcare in the rural areas is challenging and several factors have been identified to play a role, where resource availabil-

ity constituting one of the main ones. However, a simple redistribution of the resources does not resolve the issue, as certain there are other barriers hindering the improvement including rural patients' reluctance to seek help, distance from the services, absence of transport to access services, insufficient number of healthcare professionals, as well as financial constraints [1–3].

Global disparity

The paper on the rural health infrastructure in United States has evaluated the efforts to improve the disparity; however, as previously highlighted, much remains to be done, particularly in terms of the smallest and very remote areas which have been mostly affected [3]. A study in Ghana highlighted illiteracy and language barrier as factors which limit rural healthcare provision in their environment [4]. Furthermore, in India, large disparities were the underlying cause of the change, and in 2016 and 2018 the main emphasis was focused on strengthening the primary healthcare system as well as on improving the access to the secondary and tertiary services by introducing nearly universal health insurance scheme [5]. Nevertheless, despite the problem recognition and the efforts taken, health disparity still remains a global concern. As mentioned in the previous section, a number of factors contribute to the aforementioned disparity, although they vary in degree – depending on whether a higher or lower income country is evaluated. In a number of cases, the rural areas experience a shortage of qualified staff, as the personnel might be unwilling to live and work in a more remote area with fewer amenities. In order to circumvent this problem, incentives should be offered which would attract and retain staff in the rural environment. The type of the incentive, however, will vary depending on the country's socio-economic development, with enforced legal contracts and mandatory rural bonded scholarships playing a more crucial role in countries, such as Australia and Japan, whereas in Cambodia and Laos financial incentives are more frequent [6].

Approaches to reduce disparity in healthcare

Traditionally, healthcare systems worldwide adopt a top-down approach which is initiated by large institutions providing resources required for large scale projects, along with the centralisation of efforts. Although it does lead to a change, the results can be only temporary. In fact, a top-down approach can result in little engagement and lack of ownership on the front line. Furthermore, since it does not address the underlying behaviour of a community, it often tends to leave the project unsustainable in the long term. A different approach is a bottom-up initiative which aims to achieve a change on a local level by involving the community, as it empowers the people to drive the project which gives more sustainable results [7].

To discuss the issue of healthcare access and to review the success of any approach to offset poor health outcomes, the authors of this paper use an example of a bottom-up initiative implemented in rural India. We provide a review of an institution established in a lower-middle income country, as a case study in order to draw parallels with other systems implemented world-wide, as well as to derive universally applicable conclusions.

Bottom-up approach in Gudalur Valley

Gudalur is a municipality situated in Tamil Nadu state in South India. According to the latest classification of the World Bank, India belongs to the lower-middle GNI per capita group [8]. It is the second most populated country in the world (with the estimated population of 1.4 billion), experiencing both rapid economic growth and significant overpopulation [9]. Technically, India's healthcare system is free to all citizens and the public sector, i.e. Government Hospitals offer free healthcare at the point of use. Unfortunately, due to staff and equipment shortages reported in the government-run healthcare, many people turn to the private sector. Nevertheless, efforts are underway to reduce these disparities by implementing programmes, such as universal sanitation coverage, the provision of clean cooking fuel, as well as vaccinations for children under age of 2 and many others [10]. Nonetheless, the limited resources constitute a contributing factor to the many health problems faced by the population. To address health disparities in the rural areas, the 2018 centralised budget strategy 'Ayushman Bharat' was focused on strengthening the primary healthcare system and on the introduction

of a nearly universal health insurance to improve the secondary and tertiary care access [5]. Despite the efforts of the government to improve India's healthcare in recent decades, less than 5% of gross domestic product has been dedicated to health expenditures [10].

The Gudalur Valley is home to more than 20,000 Adivasis (tribal people) who until recently had no access to healthcare, as they have been unable to easily access government-ran health services. Therefore, a charitable organization referred to as ASHWINI, The Association for Health Welfare in the Nilgiris, was established to improve the service provision and to decrease Adivasis' mortality rate. The association was founded in 1990, and its aim has always been to achieve 'an accessible, effective and sustainable health system that is owned by the community' [11]. The project started with the focus to address infectious diseases, malnutrition and both maternal and infant mortality among the vulnerable Adivasi population living in Nilgiri mountains in South India. The approach adopted by the founders focused on employing locals as staff, as well as on encouraging the community's participation in the decision-making process in order for the tribal team to take over the management. This sustainable and people-focused initiative grew and evolved into a hospital which caters to over 20000 Adivasis, with 8 accessible community based centres. The constant development has led to the opening of a nursing school, thus allowing the tribal people to enrol in studies, train in dentistry and involve in other courses, such as administration and physiotherapy. Furthermore, ASHWINI encourages international links, and their medical elective programme has been beneficial for both visitors and hosts, increasing the Adivasis' connection to the world [11]. The relationship between selected aspects of healthcare and the bottom-up approach employed in the ASHWINI model is discussed in the sections below.

Malnutrition

Malnutrition in India continues to be a recognised problem. In 1975 the Government of India introduced Integrated Child Development Services (ICDS) – a programme aiming to deliver healthcare, nutrition benefits, health follow-ups, immunisations and other services for children up to six years old, as well as for pregnant and breastfeeding women. These basic healthcare needs were to be provided by health centres, situated all over India. However, in 2015–2016 The National Family Health Survey in India highlighted that 1 in 2 children still suffered from nutritional deficiencies. In fact, one of the ICDS's pitfalls, particularly in terms of tribal communities, was the geographical inaccessibility of health centres [12].

Although less than 50% of the population were of normal weight, only a small proportion sought help, presumably partly due to the geographical inaccessibility, as mentioned above. The alarming figures have been the reason behind ASHWINI's outreach programme which has been training community health workers who visit tribal hamlets, assess growth of children under the age of 5 and identify patients who need nutrition benefits. In spite of the fact that 45% of the local population suffers moderate to severe malnutrition, between 2017–2018, 92.3% of all children had their growth monitored, and 595 children benefited from the ICDS programme [13].

Antenatal care, maternal and infant mortality among the Adivasis

Taking into consideration the fact that, according to data collected from a few villages, infant mortality rate in the area was as high as 250 per 1000 in 1988, and several instances of maternal death were recorded, ASHWINI's initial focus was to address maternal health, antenatal and postnatal care. Both high maternal (145 deaths per 100 000 live births) and high infant mortality rates (39.55 deaths per 1000 live births) constitute India's national problem [9]. Similarly to the case of malnutrition, the figures tend to be greater among the tribal communities, due to physical barriers in accessing healthcare, unsanitary conditions and poor nutritional, and hence, physiological reserve. ASHWINI owes its success to the outreach programme where health volunteers identify pregnant women in the community and monitor their progress until the delivery. In years 2017–2018, 296 tribal deliveries occurred with 91.5% of women having > 3 antenatal follow-ups, and only 1 maternal death was noted. Nevertheless, although infant mortality rate decreased, it still remains at 21.2%. In contrast to the out-of-hospital midwife-led deliveries encouraged in the West, a strong shift towards inpatient deliveries was observed in ASHWINI. In

the period of 2017–2018, 94.9% of deliveries took place in hospital, ensuring access to the sanitary conditions as well as to trained staff and necessary equipment in case of complications [13].

Mental Health and use of substances

Due to poverty and challenging living conditions, many Adivasis suffer mental health problems. ASHWINI established Mental Health Programme which between 2017–2018 identified 62 new patients suffering from mental illnesses and provided 259 patients with the treatment for their mental health condition [13].

Additionally, despite a limited access to alcohol, alcohol abuse was noted, mainly among men. Many Adivasis were also seen consuming betel nut, which has been linked to cancer. Healthcare staff have been trying to discourage the consumption of either of the substances, since they affect health negatively. This, however, comprises a difficult endeavour, due to the fact that both alcohol and betel nut are employed as a coping mechanism which helps to deal with the hardship, hunger and poverty.

Comparing ASHWINI to other healthcare models used worldwide

Discussing India's approach to healthcare deserves a separate analysis due to the population size, cultural and religious diversity. Notwithstanding the existence of a national public healthcare system, there is some diversity in the implementation and execution of health-related policies between the states, and even within the states themselves. Therefore, the analysis of the approach which aims to improve healthcare in the rural areas requires taking into account the political system of the country. India on the whole is a democratic country, i.e. its political system is generally similar to that in Northern America and the European countries [14]. This distinction renders India unique among other lower-middle income countries, which largely have a degree of authoritarian regimes, thus making the comparative process more difficult. Furthermore, a comprehensive comparison of India's healthcare system with healthcare in other lower-middle income countries would exceed the scope of one article; hence, India remains the main focus of this paper.

Up to date, the implementation of a bottom-up approach to healthcare has not been extensively discussed in the scientific literature, therefore, the evaluation of a successful initiative in the rural region of India seems valuable. Moreover, it may be a starting point for further assessment of the effectiveness of this approach with regards to improving healthcare sustainability, by means of utilising local manpower, local infrastructure and local resources.

Poverty, combined with insufficient infrastructure, including staff, equipment and facilities are frequently at the root of poor health outcomes. The problems highlighted, faced by Adivasis in Gudalur region, are experienced universally by the rural communities worldwide. The principles of ASHWINI including bottom-up approach, empowering local community and the focus on sustainability of the results are also universal in nature, indicating they could be successfully transferred to any other environment with a similar infrastructure. In 1993, India passed the Panchayat Raj Act which assigned the responsibility of developing health plans to the individual districts, expecting that a decentralised planning system would better reflect and address the local community needs and improve resource exploitation. Nevertheless, this approach was successful in certain districts, although it failed in others. It was noted that factors, such as a lack of external input from government officials, were interpreted as a lack of interest, contrary to the assumption, and thus resulted in little community involvement. The district where the state offered supervision and periodically requested reports coped better, and the local staff felt safe and encouraged. This demonstrates that locally managed services supervised by their superiors might achieve better results compared to a top-down or a bottom-up approach only [15].

In Uganda, a randomised experiment was performed which aimed to improve quality and quantity of service provision by encouraging the community to monitor local healthcare providers. The study found that community's involvement in monitoring service delivery was reflected in improved staff accountability, and increased staff efforts to serve the local population. This

resulted in better service provision where the traditional top-down supervision has failed [16].

In Garissa District Hospital in Kenya a hybrid model of service delivery has been employed. National level ministry personnel pays regular visits to provide supervision and ensure care standards, whereas hospital management teams with the community leaders on the hospital board and continue to identify and solve problems which appear locally. The success of this model is reflected in the figures – inpatient admissions increased by 33%, the number of self-discharging patients decreased by 90%, number of deliveries increased by 50%, while the new-born death rate decreased by 75% [17].

Taking a brief look at higher income countries, one of the quoted barriers to improving National Health Service (NHS) in the UK, is that the commissioner's vision of improvement is not shared by the organisations providing the services, thus they may not feel motivated to achieve better outcomes at a local level [18]. Therefore, little engagement on the front line is universally one of the main pitfalls of a top-down approach. In the Netherlands, healthcare is also regulated top-down [19], whereas in Switzerland bottom-up approach has been adopted. Switzerland has delegated the responsibility for the efficient medical care to the GPs, and it has transpired that the doctors participating in the scheme have shown a lot of initiative for providing quality, cost-effective service [19]. In contrast, in Scotland and Ireland, quality improvement efforts are supported by organisations which employ a hybrid model where selected components of both top-down and bottom-up approaches are implemented [20]. The abovementioned examples demonstrate that the approach to organising healthcare services varies between countries. However, it is claimed that certain features of a top-down approach, such as central coordination and pooled resources, once combined with a bottom-up ownership and engagement, can result in a successfully implemented change [7].

Conclusions

A bottom-up approach applied by ASHWINI has improved health outcomes in the area by introducing child and antenatal care, offsetting malnutrition and addressing mental illness. ASHWINI's approach is based on universal principles, therefore, if applied in other rural areas with a similar demographic, resource and infrastructural environment, it may lead to improved healthcare outcomes.

Employing a bottom-up approach, preferably alongside the selected components of a top-down system, is essential if healthcare outcomes are to improve and the change is to be long-lived.

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

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ORIGINAL PAPER

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Changes in weight and body composition after initiating insulin therapy and their relationship with the metabolic control during the first year of type 1 diabetes in adults. InLipoDiab1 Study

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ABSTRACT

Introduction. As in the general population, an increase in the incidence of overweight has been observed in individuals with type 1 diabetes (T1DM). Nevertheless, weight gain in this group may contribute to the deterioration of the metabolic management. The aim of this study was to evaluate changes in the body weight and body composition after initiating insulin therapy and to assess their relationship with the metabolic management during the first year of T1DM in adults.

Material and Methods. The prospective analysis included 139 adults patients with newly diagnosed T1DM, treated with Intensive functional insulin therapy (IFI) from the onset of the disease (age 26.3 ± 5.9 years). Patients were assessed at the time of the diagnosis and after 12 months. Metabolic parameters, including the HbA1c and lipid profile were investigated. The group was divided according to weight gain during the follow-up period.

Results. Weight gain was observed in 68.3% of participants (n = 95). In most cases an increase in body fat was found (41% vs 59% p = 0.01). Changes in the body weight corresponded to significant changes in body composition. Conversely, HbA1c decreased during the follow-up in all groups. The highest reduction was observed in a group with "excessive weight gain". Additionally, a significant increase in high density lipoproteins was observed in each group. However, weight gain was not accompanied by a deterioration of the lipid profile.

Conclusions. Weight gain is a considerable problem among adults with newly diagnosed T1DM and is connected mainly with an increase of adipose tissue above the normal range. Changes in the body weight, associated with body composition changes, did not result in the dysfunctions of the metabolic management.

Introduction

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease characterized by absolute insulin deficiency. Furthermore, an increase in the incidence of overweight has been observed in individuals with T1DM [1]. In turn, excessive body weight (BW) and weight gain increase cardiovascular events and may contribute to the deterioration of the metabolic management, including lipid profile [2, 3].

Intensive functional insulin therapy (IFI) constitutes the treatment of choice in T1DM [4], where one of the side effects is an increase of BW [5]. In fact, beneficial changes in lipid parameters have been observed in the first months following the implementation of Intensive functional insulin therapy (IFI) [6]. However, it has been noted that a significant increase of BW as well as its time duration may suppress the beneficial effects of IFI [3].

Moreover, during the first year after implementing IFI, BW and body composition changed significantly, thus, monitoring of these changes might be necessary [8]. To date, there has been no prospective study analysing the changes in anthropometric parameters and body composition, as well as their relationship with the metabolic management in adults presenting with T1DM.

The aim of this study was to evaluate changes in the body weight and body composition after initiating IFI and their relationship with the metabolic parameters provided in the recommendations of Diabetes Poland [9], including the level of glycated haemoglobin (HbA1c) and lipid profile during the first year of T1DM in adults.

Material and Methods

The prospective analysis included 139 patients with newly diagnosed T1DM (42 women, mean age 26.3 ± 5.9 years), participants of the still ongoing Insulin Therapy and Lipoproteins Profile in Type 1 Diabetes Study (InLipoDiab1, NCT02306005). The study was approved by an appropriate bioethics committee, and the patients provided their written informed consent to participate in the study.

Exclusion criteria included age under 18 and above 35 years, presence of comorbidities and medication use for other disorders than T1DM, a lack of written consent, a lack of data at two control points.

The autoimmune aetiology was confirmed in all patients by the positive specific autoantibodies. The patients were treated with IFI using insulin pens from the onset of the disease. A description of the methodology has been outlined in the previous study [6].

Anthropometric data, including height, waist, and hip circumference, were measured by trained researchers using standardized meter measures. BW, fat mass (FM) [%], free-fat mass (FFM) [kg], total body water (TBW) [l] content were measured by Body Composition Analyzer Tanita BC-418. The body mass index (BMI) and waist-to-hip ratio (WHR) were calculated on the basis of the following formulas: BMI = weight (kg)/squared height (m^2) and WHR = waist circumference (cm)/hip circumference (cm), respectively. The content of the adipose tissue was evaluated according to the Tanita scale.

Data with regard to a self-reported weight loss and the body weight prior to the diagnosis were provided by the participants and collected during the interview at baseline.

The daily dose of insulin (DDI) was defined as the requirement for insulin per kilogram body weight per day. This amount of insulin was calculated as the sum of units of long- and short-acting insulin. The final DDI at the time of the diagnosis was established on the last day of hospitalization when glucose levels reached the treatment target, and the patient could be discharged home. The DDI following one year since the diagnosis was based on the data derived from patients' self-monitoring logs from the previous month.

The lipid profile, including the levels of TC, HDL, and triglycerides, was measured using a Cobas 6000 biochemistry analyser (Roche Diagnostics, Basel, Switzerland), by means of enzymatic colorimetric methods. Low-density lipoprotein levels were calculated by the Friedewald formula, whereas glycated haemoglobin (HbA1c) concentration was assessed by a turbidimetric inhibition immunoassay (Cobas 6000, Roche Diagnostics).

The study group was divided according to the extent of change in BW expressed in percentages during the first year of T1DM into 2 subgroups:

$$\%\ \text{BW change} = \frac{\Delta BW\ after\ 1\ year\ [kg]}{baseline\ BW\ [kg]} * 100\%$$

The change expressed only as kilograms may be misleading and does not comprise the differentiation in initial BW, as well as the sex-related differences [7]. Therefore, the following thresholds were adopted:

- Group 1 – weight loss (change in initial BW < 0%); n = 41 (29.5%).
- Group 2 – minimal weight gain (change in initial BW from 0 to ≤5%); n = 43 (30.9%).
- Group 3 – excessive weight gain (change in initial BW > 5%); n = 55 (39.6%).

Statistical analysis

The statistical analysis was performed using STATISTICA, version 13 (StatSoft, Inc, Tulsa, OK, USA). Only the participants with the set of data at two control points were included in the statistical analysis. All data were presented as median values and interquartile ranges (IQRs), and the number (percentage) of patients. First, the data at baseline and follow-up were compared, then, participants were divided into three groups. The normality of data distribution was tested using the Shapiro-Wilk test, and the comparative analysis of three independent groups was performed using the Kruskal-Wallis test. When statistically significant differences occurred, an appropriate posthoc test was applied (Bonferroni, T2 Tamhane's test). In comparison of these two time periods, the student's t-test for dependent samples, or its non-parametric equivalent, i.e., the Wilcoxon test, was used. Moreover, p-value < 0.05 was considered statistically significant.

Results

1. Baseline

1.1. Characteristic of the study population

The study involved 139 individuals, with 41 (29.5%) included in the weight loss group (1), 43 (30.9%) were classified to the minimal weight gain group (2), and 55 (39.6%) – to the excessive weight gain group (3). The compared groups were equal in number, p = 0.29. Characteristics are presented in **Table 1**.

Table 1. Characteristics of the research group. The anthropometric data and body composition at baseline and during the follow-up

		Weight loss (1)	Minimal weight gain (2)	Excessive weight gain (3)	p-value
Sex, n (%)	Female	10 (24.4)	16 (37.2)	16 (29.1)	p = 0.43
	Male	31 (75.6)	27 (62.8)	39 (70.9)	
Increase in body fat, n (%)		8 (20.5)	27 (67.5)	47 (85.5)	p < 0.001
Body weight, kg	Baseline	76.6 (66.5–82.5)	68.3 (57.8–77.8)	67.9 (56.8–78.5)	p = 0.06
	Follow-up	72.9 (63.5–77.3)	70.5 (59.2–78.9)	76.3 (64.0–85.4)	p = 0.29
Bodyweight before the diagnosis, kg		83.3 (70.2–87.5)	75.9 (67.2 – 84.3)	76.25 (63.8 -89.8)	p = 0.26
Self-reported weight loss, kg		-5.0 (-10.0–(-3.8))	-6.0 (-10.0-(-4))	-7.5 (-10.0 – (-5.0))	p = 0.16
BMI, kg/m^2	Baseline	23.1 (21.6–26.3)	22.4 (20.1–24.2)	22.0 (19.4–24.6)	p = 0.1
	Follow-up	22.1 (20.4–25.3)	22.7 (20.6–24.9)	23.8 (21.5–26.7)	p = 0.04$_c$
WHR	Baseline	0.9 (0.8–0.9)	0.8 (0.8–0.9)	0.8 (0.8–0.9)	p = 0.28
	Follow-up	0.8 (0.8–0.9)	0.8 (0.8–0.8)	0.8 (0.8–0.9)	p = 0.69
Waist circumferences, cm	Baseline	87.0 (81.0–91.0)	80.0 (72.0–85.0)	80.0 (73.0–90.0)	p = 0.04$_a$
	Follow-up	80.0 (74.0–84.0)	80.5 (75.0–85.0)	81.0 (74.0–93.0)	p = 0.53
Fat mass, %	Baseline	18.0 (12.0–23.5)	19.1 (12.4–22.5)	14.4 (7.5–21.3)	p = 0.07
	Follow-up	12.7 (9.6–18.1)	18.8 (12.15–22.9)	18.4 (12.7–26.0)	p = 0.009$_c$
Free-fat mass, kg	Baseline	62.8 (54.9–67.3)	59.15 (44.0–66.2)	59.7 (45.4–66.0)	p = 0.25
	Follow-up	62.8 (54.7–66.7)	60.5 (43.1–67.9)	63.0 (46.0–69.7)	p = 0.57
Total body water, l	Baseline	45.9 (40.2–49.3)	43.3 (32.5–48.5)	43.7 (33.5–48.3)	p = 0.3
	Follow-up	44.1 (40.0–48.8)	44.3 (31.6–49.7)	46.5 (33.7–51.0)	p = 0.55

Abbreviations: BMI, body mass index; WHR, wait-to-hip ratio.
Gender, education, body fat, smoking status, physical activity according to the weight changes status was performed using chi-square test. Comparative analysis of anthropometric data and body composition at baseline and the follow-up by weight change group was performed using the Kruskal-Wallis test. The results are presented as median and interquartile range.

$_a$P < 0.05 Weight loss vs. Minimal weight gain
$_b$P < 0.05 Minimal weight gain vs. Excessive weight gain
$_c$P < 0.05 Weight loss vs. Excessive weight gain

1.2. Anthropometric and body composition at baseline

Anthropometric and body composition data at onset are listed in **Table 1**. Statistically significant differences regarding anthropometric and body composition variables at onset were observed only for waist circumference between groups 1 and 2.

1.3. Metabolic management at baseline

Data regarding the metabolic control in each group are presented in **Table 2**. Significant differences at baseline were applicable only to HbA1c. Furthermore, neither lipid parameters nor DDI differed significantly between the groups at the onset.

2. Data at the follow-up (after 12 months from onset)

2.1. Anthropometric data and body composition at the follow-up

After one year from the diagnosis compared to baseline, a higher percentage of patients from group 3 presented an increased adipose tissue content above the normal range (p = 0.02) (9.1% vs. 25.5%) (**Figure 1**). Additionally, following one year from the diagnosis, statistically significant differences related to the median BMI and body fat content were observed (**Table 1**).

2.2. Metabolic management at the follow-up

Statistically significant metabolic management differences were not observed after one year from the onset between the three groups (**Table 2**).

3. Changes during 12 months of observation

3.1. Changes in anthropometric parameters and body composition

The comparative analysis between the three groups is presented in **Table 3**.

WHR in group 1 decreased (P = 0.001), whereas in other groups no statistically significant differences were found. Moreover, waist circumference in group 1 decreased significantly (P < 0.001) and increased in group 3. In group 1, the median FFM was reduced (P = 0.02) and a significant increase of the median FFM in groups 2 (P = 0.008) and 3 (P < 0.001) was observed. In addition, in group 1, TBW decreased with statistical significance (P = 0.01). In the course of the observation period, TBW significantly increased among groups 2 (P = 0.007) and 3 (P < 0.001). Moreover, FM correlated with weight change in groups 1 and 3, whereas in group 1, the median content of the adi-

Table 2. Comparison of the metabolic management at baseline and the follow-up according to weight change groups

		Weight loss (1)	Minimal weight gain (2)	Excessive weight gain (3)	p-value
HbA1c, %	Baseline	10.10 (9.5–11.6)	10.5 (9.5–12.1)	12.1 (11.0–12.8)	p < 0.001[bc]
	Follow-up	6.6 (5.8–7.9)	6.5 (6.1–7.3)	6.8 (6.2–7.7)	p = 0.58
HbA1c, mmol/l	Baseline	87 (80–103)	91 (80–109)	109 (97–116)	p < 0.001[bc]
	Follow-up	49 (39–63)	48 (43–56)	51 (44–61)	p = 0.59
TC, mmol/l	Baseline	4.5 (3.6–5.3)	4.3 (3.9–4.7)	4.3 (3.7–5.0)	p = 0.89
	Follow-up	4.8 (3.8–5.0)	4.5 (3.9–5.1)	4.5 (3.9–5.2)	p = 0.87
HDL, mmol/l	Baseline	1.2 (0.8–1.4)	1.2 (0.9–1.5)	1.2 (1.0–1.3)	p = 0.85
	Follow-up	1.7 (1.5–2.0)	1.9 (1.5–2.3)	1.7 (1.4–2.1)	p = 0.16
LDL, mmol/l	Baseline	2.5 (1.7–3.4)	2.5 (2.1–2.9)	2.5 (2.0–3.1)	p = 0.81
	Follow-up	2.3 (1.7–2.7)	2.1 (1.7–2.6)	2.3 (1.6–2.9)	p = 0.8
TG, mmol/l	Baseline	1.1 (0.9–1.7)	1.1 (0.9–1.8)	1.3 (0.9–1.3)	p = 0.74
	Follow-up	0.9 (0.6–1.6)	0.7 (0.5–1.1)	1.0 (0.7–1.2)	p = 0.26
DDI, IU/day	Baseline	0.1 (0.1 – 0.2)	0.1 (0.1–0.3)	0.2 (0.1 – 0.4)	p = 0.35
	Follow-up	0.3 (0.2 – 0.6)	0.3 (0.2 – 0.4)	0.3 (0.2 - 0.5)	p = 0.76

Abbreviations: DDI, daily dose of insulin; HbA1c, glycated haemoglobin; HDL, high density lipoprotein; LDL, low density lipoprotein; TC, total cholesterol; TG, triglyceride.
The results are presented as median and interquartile range. Comparative analysis was performed using the Kruskal-Wallis test.

[a] P < 0.05 Weight loss vs. Minimal weight gain
[b] P < 0.05 Minimal weight gain vs. Excessive weight gain
[c] P < 0.05 Weight loss vs. Excessive weight gain

pose tissue significantly decreased (P < 0.001). However, the opposite effect was observed in group 3 (P < 0.001).

3.2. Changes in the metabolic management

In each group, the median level of HbA1c decreased significantly (p < 0.001), although the reduction of HbA1c was greater in group 3 than in group 1 (p = 0.002) (**Table 3**). The annual distributions of HbA1c in each group are shown in **Figure 2**.

In each group, the median TG level decreased significantly (for group 1 p = 0.01; for group 2 and 3 p< 0.001). Additionally, although no statistically significant differences were observed between the two time periods in either group regarding TC, a significant reduction in LDL in groups 2

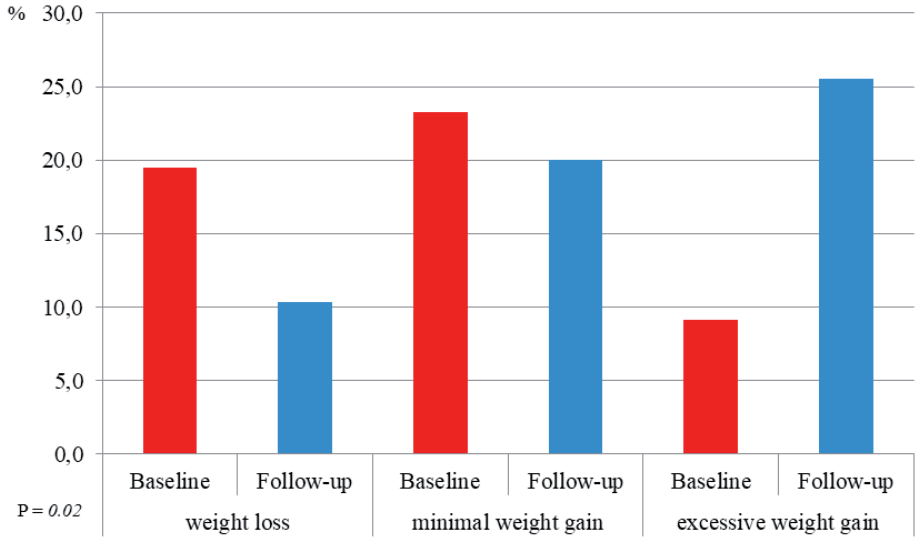

Figure 1. Percentages of subjects with the adipose tissue above normal in relation to weight gain group baseline and at follow up

Table 3. Changes in the anthropometric data, body composition, and the metabolic management by weight change group during the follow-up

	Weight loss (1)	Minimal weight gain (2)	Excessive weight gain (3)	P-value
▲Body weight, kg	-3.2 (-5.3–(-1.2))	1.9 (0.9–2.4)	6.6 (4.7–9.6)	p < 0.001 [abc]
%change in body weight, %	-4.5 (-6.9–(-1.8))	2.8 (1.3–4.0)	9.4 (6.6–14.1)	p < 0.001 [abc]
▲BMI, kg/m^2	-1.1 (-1.7–(-0.4))	0.7 (0.3–0.8)	2.09 (1.5–3.01)	p < 0.001 [abc]
▲WHR	-0.07 (-0.8–(-0.0))	-0.02 (-0.69–0.0)	-0.02 (-0.8–0.01)	p = 0.39
▲Waist circumferences, cm	-9.0 (-79.0–(-2.0))	0.0 (-64–5.0)	-1.0 (-74.0–5.0)	p = 0.05
▲Fat mass, %	-2.5 (-5.1–(-0.4))	0.75 (-0.6–2.7)	4.4 (1.5–6.7)	p < 0.001 [abc]
▲Free-fat mass, kg	-0.5 (-1.4–0.2)	0.6 (-0.5–1.6)	2.5 (0.6–4.5)	p < 0.001 [abc]
▲Total body water, l	-0.5 (-1.2–0.0)	0.4 (-0.3–1.2)	1.8 (0.2–3.9)	p < 0.001 [ac]
▲ HbA1c, %	-3.7 (-4.7–(-1.5))	-4.1 (-6.0–(-2.4))	-5.0 (7.1–(-2.7))	p = 0.002 [c]
▲ HbA1c, mmol/l	-36 (-47–(-16))	-47 (-66–(-25))	-55 (-74–(-36))	p = 0.002 [c]
▲TC, mmol/l	0.36 (-0.2–0.9)	0.03 (-0.6–0.8)	-0.01 (-0.4–0.4)	p = 0.18
▲HDL, mmol/l	0.6 (0.4–0.8)	0.1 (0.4–1.0)	0.5 (0.2–0.9)	p = 0.35
▲LDL, mmol/l	-0.1 (-0.7–0.2)	-0.5 (-1.0–0.2)	-0.3 (-0.6–0.3)	p = 0.75
▲TG, mmol/l	-0.3 (-0.6–0.1)	-0.5 (-1.0–(-0.2))	-0.3 (-0.6–(-0.1))	p = 0.48

Abbreviations: BMI, body mass index; WHR, wait-to-hip ratio; HbA1c, glycated haemoglobin; TC, total cholesterol ;HDL, high density lipoprotein; LDL, low density lipoprotein ; TG, triglyceride.

The results are presented as median and interquartile range.
Comparative analysis was performed using the Kruskal-Wallis test.
▲; for example, ▲ body weight was defined as the difference between the body weight at the follow-up [kg] and onset [kg].

[a] P < 0.05 Weight loss vs. Minimal weight gain
[b] P < 0.05 Minimal weight gain vs. Excessive weight gain
[c] P < 0.05 Weight loss vs. Excessive weight gain

(p = 0.005) and 3 (p = 0.002) was demonstrated. Furthermore, in each group, a significant increase in HDL was observed (p < 0.001); the lowest increase was shown in group 3, while the highest was found in group 2. The annual distributions of HDL in each group are shown in **Figure 3**. However, no differences were observed between groups with regard to the changes in lipid profile according to weight gain (**Table 2** and **3**).

Discussion

The study results clearly indicated that BW changes following T1DM diagnosis. Weight gain, observed in 68.3% of participants, was mainly related to an FM increase and increased adipose tissue above the normal range. Moreover, changes in BW correspond to significant changes in body composition (TBW, FM, FFM). Nevertheless,

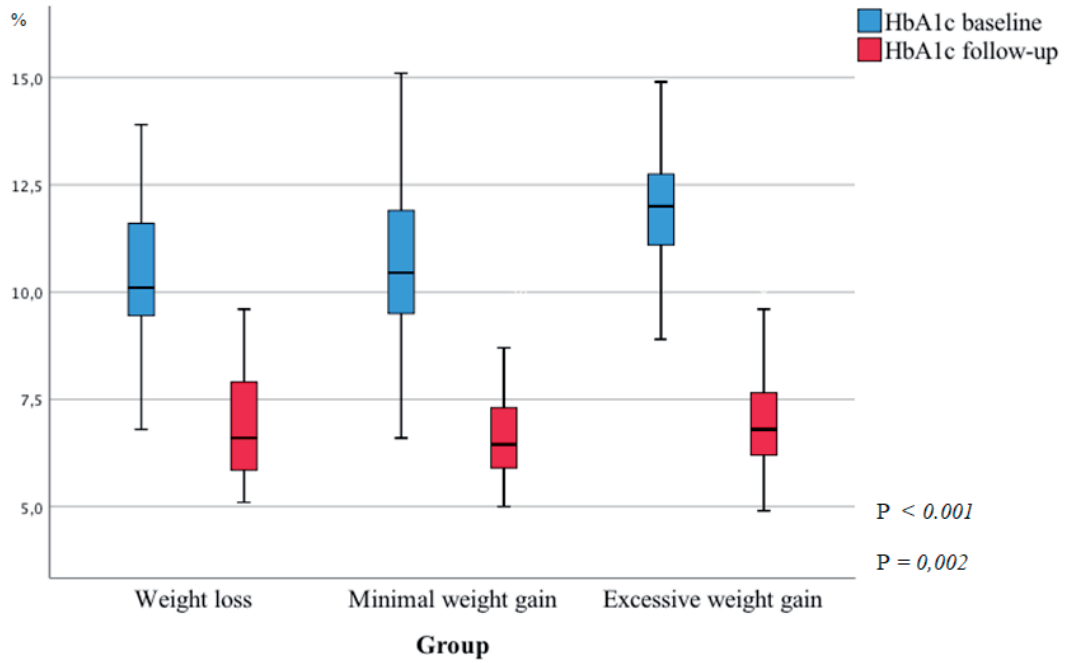

Figure 2. The annual distributions of HbA1c in each group

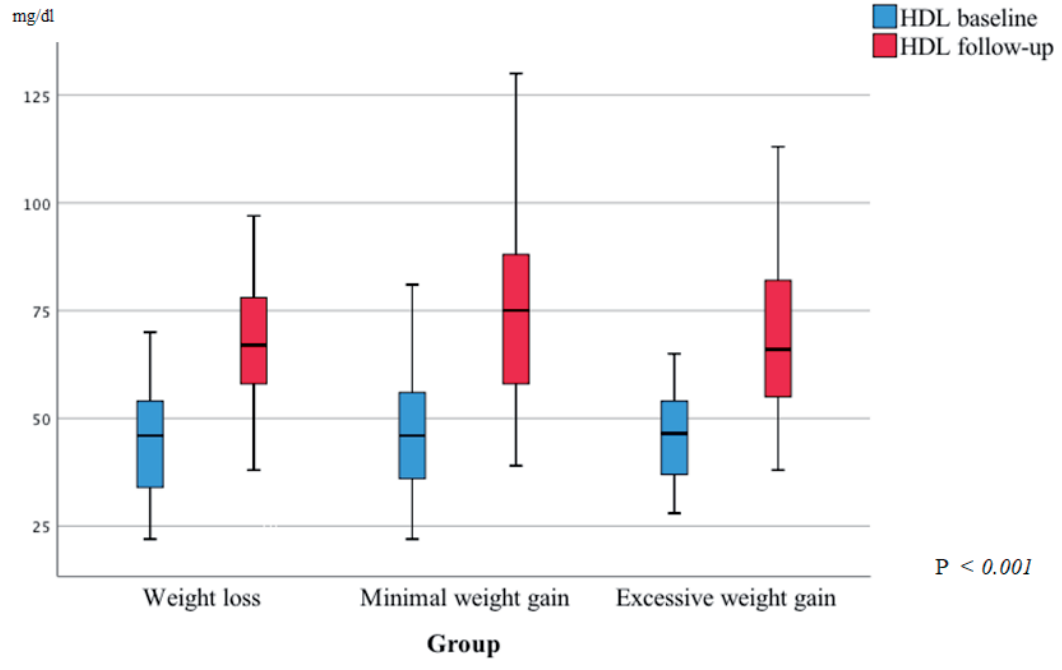

Figure 3. The annual distributions of HDL in each group

the available literature with regard to BW, body composition and their association with the metabolic management in adults with newly diagnosed T1DM is scarce.

Weight gain among the participants of the Diabetes Control and Complications Trial (DCCT) was increased most rapidly within the first year of observation (an average of 3.3 kg). It was mainly related to an increase of FFM [10], and progressed with time [2]. However, in DCCT, the follow-up period did not commence at the onset of the disease.

According to the study by Carlson and Campbell [12], an FM increase accounted for 2.4 of the 2.6 kg of increased weight.

The lack of FFM changes can be accounted for by the small study population (n = 6) and the short duration of the follow-up (2 months). In turn, other observational studies, also with the participation of a small study population (n = 10), demonstrated that during the first year after the onset of T1DM the mean increase in BW is 6.5% (4.3 ± 2.9kg), with a simultaneous increase in FM and FFM [8].

The participants in our study whose BW decreased during observation, also experienced a significant decrease in FM, FFM, and TBW, whereas TBW as part of FM was found to increase with an increase in fat content [13].

The adipose tissue, including the visceral adipose tissue, is an independent predictor of insulin sensitivity and a critical factor modulating lipid and glucose homeostasis. In individuals with T1DM, the influence on insulin sensitivity is revealed by BMI, TG, waist circumference, visceral adipose tissue, and total FM [14]. Therefore, it is crucial to monitor BW and body composition among adults with newly diagnosed T1DM in terms of the development of insulin resistance and progression. It seems particularly essential, since a decreased insulin sensitivity is a well-known risk factor for complications in this group [15].

Insulin deficiency, as a catabolic state, is correlated with a loss of nitrogen and FFM. As the previous study revealed, the pre-diagnosis unintentional weight loss as high as 6.3 ± 2.5 kg may affect body composition differently depending on the course of the disease and the changes which occur prior to the diagnosis. In fact, weight loss before the diagnosis may affect FM and FFM [8]. Therefore, the baseline body composition appears to be essential and may largely determine the subsequent metabolic management.

Although the initial BW and BMI did not differ significantly between the groups, it is noteworthy that among individuals whose weight significantly decreased, both BW and BMI were higher at the time of the diagnosis than in patients whose BW increased. This observation is consistent with the findings of Kim et al., wherein persons who lost weight presented a BMI > 25 kg/m^2 more frequently [16]. However, the most alarming observation was the fact that weight gain in such a short period of time – within only one year of observation – was primarily associated with an increase in the adipose tissue above the norm.

The second aim of the study was to analyse whether changes in BW affect the metabolic management. It turned out that the metabolic management changed significantly during the first year of the disease, although there were no significant differences between groups in terms of the level of weight gain. Nonetheless, it was observed that patients in group 1 experienced a slightly smaller reduction in LDL compared to groups 2 and 3. Each group demonstrated an increase in HDL; however, the lowest increase was in group 3. Moreover, TC increased slightly in each group, although this change was not statistically significant.

Our results are consistent with the literature. In fact, Dayem et al. [4] showed a relationship of excess BW with deteriorated lipid parameters, including significantly lower HDL, higher LDL, TC, and TG. However, these findings refer to the pediatric population and, hence, cannot be directly translated into other age groups. EURODIAB Prospective Complications Study results [11] indicate that changes in lipid profile are less favourable in the group of patients who significantly increased their BW (≥ 5 kg). A weight change one year after the T1DM diagnosis did not significantly affect the lipid profile, and the changes were only minor. Additionally, an increase of HDL occurred independently of the weight change; however, the smallest increase was observed in the group with the excessive weight gain.

It is worth bearing in mind that even though a weight gain was observed, the median BMI was within the reference range. This, in turn, emphasizes the disadvantages of BMI measurement alone, and further suggests the necessity of

monitoring the other anthropometric and body composition parameters in order to observe the clinically significant changes.

Analysing glycaemic control, HbA1c decrease was observed in the entire group. Individuals who presented a significant weight gain also demonstrated the highest reduction of HbaA1c, although simultaneously they had the highest HbA1c at the time of the diagnosis. In this respect our results are consistent with those demonstrated by Yamada et al. [17], who has suggested that neither BMI nor BW determines HbA1c among the non-obese T1DM subjects, and good control is associated with an appropriate insulin dose showing a strong correlation with BW. However, in our study, DDI did not differ significantly between groups at the onset. Therefore, weight gain observed in our study was a consequence of a lifestyle, rather than the insulin dose. The relationship between weight gain and HbA1c concentration seems to be an interesting area to explore. More favourable changes in HbA1c in group 3 can be accounted for by increased FFM. Muscle mass, a component of FFM, is involved in insulin-dependent glucose uptake, making it a vital element in maintaining carbohydrate control [18]. It has been suggested that hypoglycaemia, more frequently observed in patients who gained weight, may contribute to better HbA1c results [10, 11].

A limitation of this study is the lack of information regarding diet. However, all participants underwent the same educational course with a dietitian. Moreover, the data including physical activity and BW before the diagnosis were provided by participants. Finally, we did not investigate the rate of hypoglycaemia, and we were not able to determine body composition prior to the diagnosis.

Concluding, weight gain seems to be a considerable issue among adults with newly diagnosed T1DM, particularly since it increases the adipose tissue above the normal range. However, an increase in the body weight, associated with unfavourable changes in the body composition, did not adversely affect the metabolic management, including HbA1c and lipid profile.

Perspectives

The abovementioned changes in the body weight require careful consideration in the course of T1DM since the very beginning of the disease. Another crucial factor seems to be the initial BW at the onset and weight loss before the diagnosis. Our results clearly indicated that in adults with T1DM, weight gain was present from the onset of the disease. The recognition of this fact allows for the timely implementation of the preventive measures or the fastest possible effective therapy aiming to prevent the excessive weight gain. Moreover, the consequences of obesity are widely known [19], and are also applicable to the population of patients suffering from T1DM. The surveillance rate, despite a great progress, is still lower in T1DM as compared with the healthy individuals [20]. Thus, it is crucial to identify the factors which can improve the prognosis in T1DM. This study highlights one of such factors, and constitutes a background for future analysis and interventional studies.

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Contributions
AU conceived the idea for the study. All authors contributed to the design of the research and were involved in data collection. MM analysed the data. All authors approved the final version of the manuscript.

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

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BRIEF REPORT

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Spontaneous pneumomediastinum – a rare cause of chest pain and dyspnoe in children

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ABSTRACT

Introduction. Spontaneous pneumomediastinum is a very rare condition in children. Nevertheless it should be considered in the differential diagnosis in patients who present with chest pain and dyspnoe.
Aim. The aim of our study was to describe clinical presentation, management and outcomes of the paediatric patients with spontaneous pneumomediastinum
Material and Methods. This was a retrospective analysis of the charts of all the patients who were admitted to the Department of Pneumonology, Paediatric Allergy and Clinical Immunology in a ten year period from 01.01.2011 till 31.12.2020 in whom spontaneous pneumomediastinum was diagnosed.
Results. There were 11 children (7 females) with spontaneous pneumomediastinum. The median age of the children was 11 years (range 3 to 17.5 years). Most of the children presented to the hospital with chest pain, three children complained of the neck swelling and four children developed dyspnoe. Three children with the primary spontaneous pneumomediastinum had a history of physical exercise prior to the onset of symptoms. The secondary spontaneous pneumomediastinum occurred in two children with asthma and 4 children with pneumonia. Genetic material of human Bocavirus was identified in 3 cases. In 81.8% of children pneumomediastinum was accompanied by subcutaneous emphysema and in one case, in a child with severe pneumonia and respiratory insufficiency caused by Bocavirus with pneumorrhachis. In 10 children

computed tomography was performed, bronchoscopy in 4 and esophagoscopy in two children. There was no evidence of esophageal rupture or bronchial tree rupture in any of our patients. Three children with pneumonia and pneumomediastinum developed respiratory insufficiency, two of these were treated with mechanical ventilation and one with High Flow Nasal Cannula oxygen therapy. All the children received oxygen. In one child surgical procedure was performed and the drain was inserted into mediastinal space in order to decompress it. Outcome was favourable in all children. Mean time to recovery was 10.6 ± 1.2 days. There was no recurrence of symptoms in any of our patients.
Conclusions. Spontaneous pneumomediastinum in most cases is a benign condition, sporadically however it may progress rapidly, leading to respiratory insufficiency and warrant invasive management.

Introduction

Spontaneous pneumomediastinum is a rare condition in children defined as an accumulation of air in the mediastinal cavity that is not caused by trauma or iatrogenic causes such as positive pressure ventilation or intrathoracic surgical procedures. Spontaneous pneumomediastinum can be further divided into primary without any underlying condition or secondary which occurs in children with pulmonary conditions such as asthma or respiratory infections [1]. Primary spontaneous pneumomediastinum might be triggered by the activities leading to sudden increase in intrathoracic pressure like strenuous physical exercise, coughing, vomiting [2]. These would cause the rupture of the alveoli, and the air released from damaged alveoli in the pulmonary interstitium reaches mediastinal space along the perivascular and peribronchial sheaths [3]. The incidence varies widely from 1 in 800 to 1 in 42 000 patients presenting to emergency departments [4]. The course of the primary spontaneous pneumomediastinum is usually benign, although in many patients thorough diagnostic procedures including computed tomography scans, bronchoscopy and esophagoscopy are performed in order to exclude underlying pathologies like esophageal or bronchial rupture and avoid possible severe complications.

Aim

The aim of our study was to describe clinical presentation, management and outcomes of the paediatric patients with spontaneous pneumomediastinum.

Material and Methods

This was a retrospective single institution study. We reviewed the charts of all the patients who were admitted to the Department of Pediatric Pneumonology, Allergy and Clinical Immunology in a ten year period from 01.01.2011 till 31.12.2020 in whom spontaneous pneumomediastinum was diagnosed. Cases of traumatic or iatrogenic pneumomediastinum were excluded from our analysis. The diagnosis was based on radiological tests results (conventional x-rays, computed tomography or both). We collected data including anthropometric parameters, present comorbidities, clinical presentation, diagnostic studies (microbiology, molecular testing) test results, treatment regimens, length of hospital stay as well as presence and type of complications and outcome from the electronic patients records.

Results

Patients' characteristic

There were 11 children with spontaneous pneumomediastinum in the given period, 7 of whom were female. 7 children were inpatients and four were transferred from other hospitals. The median age of the children was 11 years (range 3 to 17.5 years). The clinical characteristic of our patients is given in the table 1. The incidence of pneumomediastinum calculated as the percentage of all emergency admissions due to respiratory causes was 0.0009% or 1 in 1145 admissions. Most of the children presented to the hospital with chest pain, three children complained of the neck swelling and four children developed dyspnoe in the course of pneumonia. The mean time from the onset of symptoms to the hospi-

Sex F/M	7/4
Age [years] (median)	11 (3-17.5)
Primary spontaneous pneumomediastinum	5 (45.4%)
Secondary spontaneous pneumomediastinum	6 (54.5%)
Symptoms	
Chest pain	7 (63.6%)
Dyspnoe	9 (81.8%)
Cough	8 (72.7%)
Neck pain	4 (36.4%)
Neck edema	3 (27.3%)
Tachycardia	8 (72.1%)
Tachypnoe	6 (54.5%)
Fever	5 (45.4%)
Abdominal pain	1 (9.1%)
Vomiting	1 (9.1%)
Coexisting pneumothorax	5 (45.4%)
Subcutaneous emphysema	9 (81.8%)

tal admission was 2.6 ± 2.1 days, most presented to the emergency department during the first 24 hours of their illness. Three children with the primary spontaneous pneumomediastinum had a history of physical exercise in the course of several days preceding the onset of symptoms. The secondary spontaneous pneumomediastinum occurred in two children with asthma and 4 children with pneumonia. One patient with asthma had upper respiratory tract infection that had triggered asthma exacerbation. The etiology of pneumonia was confirmed in all the cases: we isolated *Staphylococcus aureus* in one case, and identified genetic material of *Parainfluenzae* virus type 3 in 2 cases and human *Bocavirus* in 3 cases (in one case genetic material of both viruses was present). In 81.8% of children pneumomediastinum was accompanied by subcutaneous emphysema and in one case, in a child with severe pneumonia and respiratory insufficiency caused by Bocavirus with pneumorrhachis. One adolescent was an active tobacco smoker who admitted to smoking 3-4 cigarettes per day.

Management

In 10 children computed tomography was performed, bronchoscopy in 4 and esophagoscopy in two children. There was no evidence of esophageal rupture or bronchial tree rupture in any of our patients. Three children with pneumonia and pneumomediastinum developed respiratory insufficiency, two of these were treated with mechanical ventilation and one with High Flow Nasal Cannula oxygen therapy. Pneumomediastinum was present in these children prior to the initiation of ventilatory support. All the children received oxygen therapy. In one child surgical procedure was performed and the drain was inserted into mediastinal space in order to decompress it (**Figure 1**). Outcome was favour-

Table 1. Clinical characteristic of patients with spontaneous pneumomediastinum

Patients	Primary/secondary pneumomediastinum	Chest pain	Cough	Dyspnoe	Neck pain	Neck edema	Tachycardia	Tachypnoe	Fever	Abdominal pain or vomiting	Pneumothorax	Subcutaneous emphysema
1	Primary	+	-	+	+	+	-	+	-	-	-	+
2	Primary	+	-	+	+	-	+	+	-	-	+	+
3	Primary	+	-	-	+	+	+	-	-	-	+	+
4	Primary	+	+	+	-	-	+	+	-	-	+	-
5	Primary	+	+	-	+	-	-	-	-	-	-	+
6	Secondary (pneumonia)	-	+	+	-	-	+	+	+	+	+	+
7	Secondary (pneumonia)	-	+	+	-	-	+	+	+	-	+	+
8	Secondary (asthma)	+	+	+	-	+	+	-	-	+	-	+
9	Secondary (pneumonia)	+	+	+	-	-	+	+	+	-	-	+
10	Secondary (asthma)	-	+	+	-	-	-	-	+	-	-	-
11	Secondary (pneumonia)	-	+	+	-	-	+	-	+	-	-	+
Sum (%)	5 (45.4%) / 6 (54.5%)	7 (63.6)	8 (72.7)	9 (81.8)	4 (36.4)	3 (27.3)	8 (72.7)	6 (54.5)	5 (45.4)	2 (18.2)	5 (54.5)	9 (81.8)

able in all children. Mean time to recovery was 10.6 ± 1.2 days. In most children it was less than a week. Since recovery was defined as no residual pneumomediastinum on radiological exam this might be a bit longer than the actual recovery time, since we did not perform x-ray or CT every day. Mean length of hospital stay was 15.3 ± 5.6 days. There was no recurrence of symptoms in any of our patients.

Discussion

Spontaneous pneumomediastinum (SP) is a rare medical entity in paediatric patients, although probably it remains underdiagnosed in many cases [4]. It should be taken into consideration in children and adolescents with sudden onset chest pain or dyspnoea as well as subcutaneous emphysema. There are only few retrospective studies and several case reports on the presentation and management of SP in children [1,2,5-9].

Most cases of secondary SP developed in the course of respiratory tract infection, frequently of viral etiology. Emiralioglu and colleagues described pneumomediastinum in the course of rhinovirus, human bocavirus and respiratory syncytial virus infections [9]. Other authors reported influenza A (H1N1) and coronavirus infections complicated with SP [7,10]. In two cases pneumomediastinum was accompanied by pneumorrhachis as in one of our patients. Another cause of spontaneous pneumomediastinum, mainly in teenagers is asthma exacerbation [8]. Up to 40-50% children and adolescents with SP were diagnosed with asthma [6,11]. SP may appear during the first asthma attack in a child with no previous diagnosis [8]. Despite the high numbers of asthmatic among patients with spontaneous pneumomediastinum, their clinical course does not differ from patients without asthma [6]. In our series two of the patients were asthmatics one of whom had upper airways infection. Choking events as well as foreign body aspiration, none of which did we observe in our case series have been reported as a frequent cause of pneumomediastinum, in up to 12.6% of subjects in a retrospective cohort from Hong-Kong [5]. There are also several reports of association of SP with inhaled tobacco smoke or illicit drugs [7,8]. Only one of our patients admitted to tobacco smoking and two reported a history of physical exercise before the episode.

Most patients with primary spontaneous pneumomediastinum present with chest pain, and subcutaneous emphysema – most frequently swelling of the neck while patients with secondary spontaneous pneumomediastinum are more likely to present with dyspnoe and cough [2,6,8]. The onset of pain is acute and it may radiate to the back, shoulders or neck [4,12]. Tachycardia, tachypnoea are also present in many patients, as well as hoarsness, difficulties in swallowing and rhinolalia (nasally sounding voice resulting from the presence of air in the soft palate). In 18% of the patients mediastinal crunch or crepitations synchronous with the heart beat ("Hamman's sign") can be appreciated on auscultation over the cardiac apex and the left sternal border [8, 12,13]. Abbas and colleagues suggest that this different presentation of patients with primary and secondary spontaneous pneumomediastinum may translate into different diagnostic approach, and may guide further decisions concerning diagnostic approach [2].

In many patients, especially treated in earlier years invasive diagnostic procedures (bronchoscopy and gastroscopy) were performed in the fear that SP might be caused by rupture in the airways or esophageal perforation potentially leading to mediastinitis. Fortunately no such findings were ever present in any of the studies performed and indeed the spontaneous rupture of the esophagus in children is extremely rare [14]. In a literature review of studies including both pediatric and adult patients Morgan and colleagues found that bronchoscopy was performed in 14.6% of patients and esophagogastroscopy in 13% [15]. Similar results described Wong in his pediatric case series: esophagoscopy was performed in 13.8% of cases and revealed no abnormalities and bronchoscopy was performed in 27.6 % of patients and found foreign body in 3 children younger than 6 years with no documented choking episodes [5]. Fitzwater and colleagues performed CT scans and contrast radiographic studies in 80% of their patients with spontaneous pneumomediastinum, yet in none of these children tests results revealed an abnormality that would influence further management [6]. In recent years, especially in the emergency department setting chest ultrasound is gaining an increasing importance in the

evaluation of pneumomediastinum allowing to spare radiation [12].

Treatment is mostly conservative and comprises of bed rest, oxygen administration, management of underlying conditions including asthma or pneumonia. Kouritas and colleagues recommend oxygen therapy as it may increase gas absorption by six times, not all the authors however recommend this course of treatment advocating for further studies prior to such a recommendation is firmly instituted [4,12]. In a series of adult and pediatric patients, concomitantly with invasive diagnostic procedures aiming at excluding the possible source of mediastinitis in up to 43% of patients for the same reason broad spectrum antibiotics were instituted [15]. Invasive procedures in the management of spontaneous pneumomediastinum were reported extremely infrequently. Perna and colleagues reported on an adult patient who underwent thoracotomy for tension pneumomediastinum [16]. This was also a case in one of our patients with respiratory insufficiency in the course of pneumonia with rapidly progressing tension pneumothorax in whom chest tube into mediastinal cavity was inserted.

Fitzwater and colleagues based on their experience with the spontaneous pneumomediastinum in otherwise healthy children postulated that this group of patients can be safely treated on outpatient basis without hospital admission or any invasive diagnostic procedures [6]. Similar conclusions were published by Noorbakhsh and colleagues [1]. Both authors suggest an adoption of very conservative approach in children and adolescents with primary spontaneous pneumomediastinum. In children with underlying conditions these should be vigorously treated in order to avoid further complications.

Hospital stay in the described case series was 1-3 days [1,6] which is much shorter than in our Department. This may be due to the fact that more than half of our patients had underlying conditions that influenced hospital stay and on the other hand our policy of not discharging children who remain symptomatic. Considering the safety of home observation of such patients such policy should change in the future.

The retrospective nature of the study as well as the small number of patients constitute its main limitations. Despite the small numbers our report shed some light on the possible causes of spontaneous pneumomedistinum in children, underlying the important role of viral infections especially human Bocaviruses. It is also worth stressing that spontaneous pneumomediastinum should be considered in every child presenting to emergency department with chest pain and / or dyspnoe. Spontaneous pneumomediastinum in most cases is a benign condition, sporadically however it may progress rapidly, leading to respiratory insufficiency and warrant invasive management.

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

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BRIEF REPORT

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Dizziness as a first alarming symptom to neurological hospital admission: reasons and differentiation problem – a pilot study

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ABSTRACT

Aim. This prospective study aimed to assess the diversity of diagnoses in patients hospitalized in the neurology department, in whom the occurrence of dizziness was the presenting complaint during qualification for hospitalization, based on a joint assessment performed by a doctor and a physiotherapist and the implementation of treatment, including physiotherapy.

Material and Methods. The study included consecutive patients selected from 2155 individuals hospitalized between 2018 and 2020 in the Neurology Unit who reported dizziness as the presenting complaint.

Results. 100 patients (the mean age 58.68±16.57) were qualified for the study: 53 men (the mean age 59.47±15.44) and 47 women (the mean age 57.79±17.88). In the overwhelming number of cases, dizziness was associated with a vascular incident. However, cases of vertigo were also reported.

Conclusion. A variety of diagnoses were made in patients hospitalized in the neurological department in whom the occurrence of dizziness was the presenting complaint during qualification for hospitalization.

Introduction

One of the reasons why patients come to the Emergency Room (ED) is the first episode of dizziness [1, 2]. It is defined as an illusion of movement of the surroundings, one's own body, head, or the illusion of instability of the ground or uncertain posture. The illusion of rotational movement is characteristic of vertigo which is mainly associated with peripheral disorders, whereas the feeling of instability of the ground is attributed to central disorders [3]. Balance disorders and the fear of falling are not uncommon. It is estimated that this problem affects approximately 30% of patients over 65 years of age [4, 5]. The complaints reported by patients are subjective and heterogeneous, which makes it difficult to objectify them [6]. Although it is difficult to assess the severity of such symptoms based on medical history, they, undoubtedly, have a negative impact on patients' functioning in everyday life [7, 8]. However, an efficiently conducted diagnostic process, including general medical, ENT (Ear, Nose and Throat) and neurological examination, allows the detection of some symptoms suggesting the etiology of vertigo and/or dizziness [9, 10]. The diagnosis and treatment of vertigo of various origins are also of interest to physiotherapists.

Therefore, the main aim of this prospective study was the differentiation of dizziness and the assessment of its causes in patients hospitalized in the department of neurology.

This kind of research facilitates the expansion of knowledge about dizziness of various origins and, what is more, finding or choosing the best ways to recover functions lost after an incident.

Material and Methods

The study included 100 consecutive adult patients selected from 2155 individuals who reported dizziness and were hospitalized between 2018 and 2020 in the Department of Neurology, Faculty of Medical Sciences, Medical University of Warsaw. Patients with impaired consciousness and communication disorders were excluded. The age and sex of patients were not the criteria for inclusion in the study group during the first stage of the study (**Table 1**).

All the patients underwent the following diagnostic procedures: general neurological and neuroimaging examination. Particular attention was paid to the assessment of the cranial nerves, the presence of the cerebellar syndrome or disorders of proprioceptive sensation. The occurrence and type of nystagmus were assessed at the same time. For this purpose, Frenzel goggles were used in all patients.

The diagnostics were completed with an ENT consultation. The Dix-Hallpike maneuver was performed by a specialist to confirm benign paroxysmal positional vertigo (BPPV). A qualified physiotherapist assessed gait disturbances and posture. At the same time, static-dynamic tests were carried out, which enabled the planning of physiotherapy.

Patients included in the study also underwent procedures such as the Sensitized Romberg test and Sensory Integration Test (the assessment of the possibility of sensory processing of the provided stimuli – balance, sight, proprioceptive sensibility) performed by a physiotherapist. The patients underwent vestibular balance assessment under static conditions on a stable and unstable surface with and without vision. The Airex Balance Pad was used for this purpose, as it had been successfully adapted in research by Boonsinsukh et al. [16] in patients with a history of falls.

When assessing the patients while walking, the elements of the Dynamic Gait Index (DGI) were used to change the direction of gait and to draw attention to the stereotype of gait versus rehabilitation planning. DGI is used to assess both elderly patients at risk of falling, and patients

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
– Adult patients (regardless of gender) – Patients reporting dizziness as the presenting complaint – Patients without impaired consciousness and communication disorders	– Underage patients – Patients who do not report dizziness or report dizziness as one of the non-disturbing symptoms – Patients with impaired consciousness and communication disorders

after stroke, with Parkinson's disease, and labyrinth injury [17].

The treatment regimen depended on the cause of dizziness and the assessment of the functional status of patients according to the ICF (International Classification of Functioning, Disability and Health) classification. ICF is an international classification developed by the World Health Organization (WHO) mainly to standardize the ways of describing health and health-related conditions. It facilitates the classification of functioning and disability in relation to a health condition. As a clinical tool, it allows the assessment of health needs, selection of appropriate methods of physiotherapy and assessment of the effectiveness of such activities. ICF facilitates communication at the professional level of doctors and physiotherapists as well as healthcare professionals around the world as regards health and healthcare.

All the patients underwent pharmacotherapy and physical therapy according to the diagnosis.

The collected data were processed and analyzed using the SAS (Statistical Analysis System).

Results

Eventually, 100 patients aged 22 to 86 years were qualified for the study (4.6% of all hospitalized ones during the specified period): 53 men (the mean age 59.47 ± 15.44) and 47 women (the mean age 57.79 ± 17.88). The average age of all the

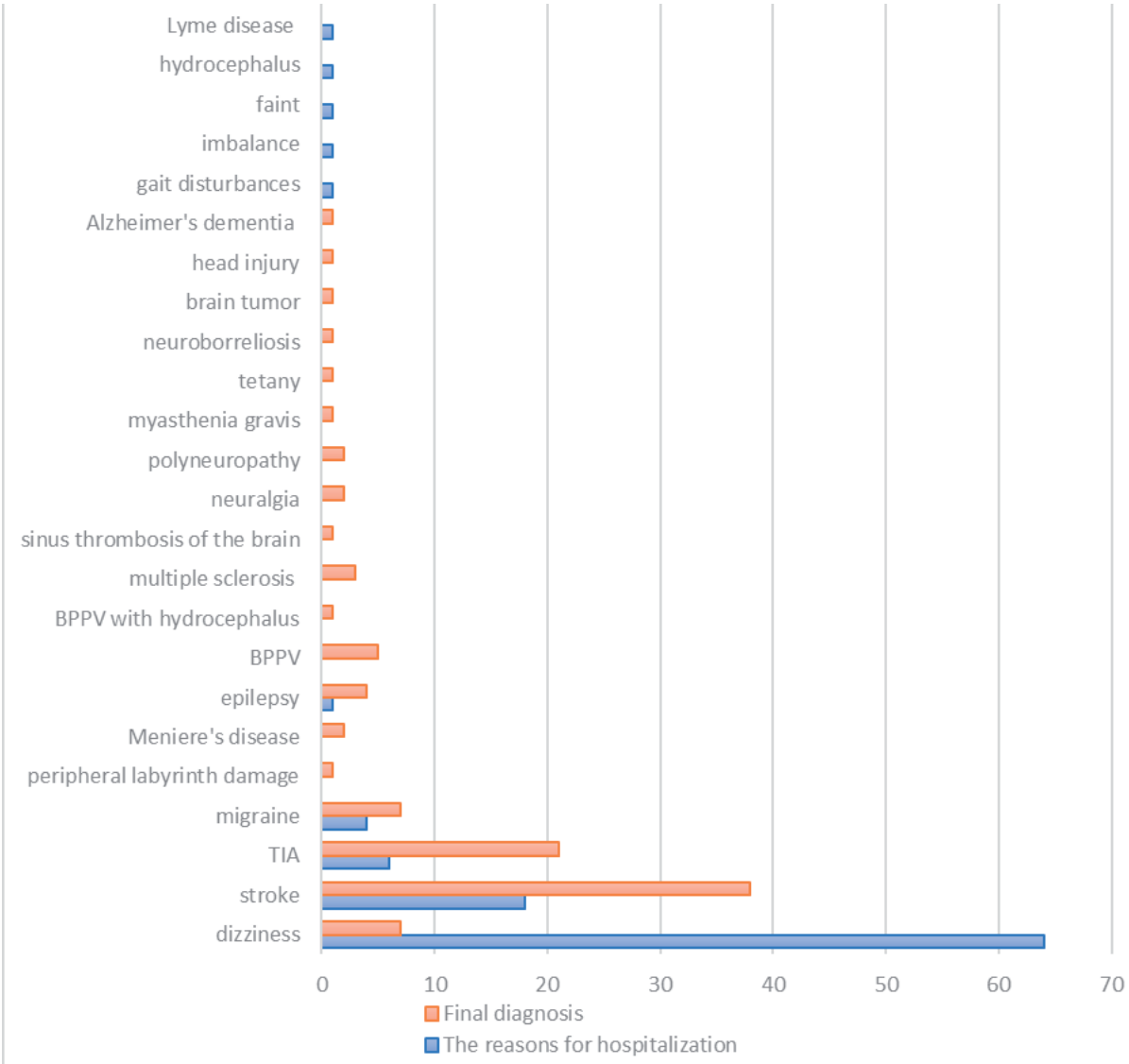

Figure 1. The reasons for the hospitalization of patients included in the study group and the final diagnoses

patients was 58.68 years (±16.57). The patients were divided into four age groups: up to 25 (4%), 26–50 (23%), 51–75, over 76 (14%) years old. The third age group (51–75 years) included 59% of the studied patients (27 women and 32 men).

The diagram presents the reasons for the hospitalization of patients included in the study group and the final diagnoses (**Figure 1**). All patients included in this study reported dizziness as the presenting complaint. The reasons for hospitalization were as follows: 61 patients with dizziness (61%) (without providing the initial diagnosis and/or the cause of the ailments described by the patients), 17 patients with stroke (17%), 4 patients with TIA (transient ischemic attack) (4%), 10 patients with headache (10%), 2 patients with gait disturbances (2%), 2 patients with weakness (2%), 1 patient with balance disturbances (1%), 1 patient with epilepsy (1%), 1 patient with Lyme disease (1%), 1 patient with hydrocephalus (1%). All the patients received the final diagnosis after an examination in the neurology department. 4 main diagnoses were made: stroke – 34 patients (34%), TIA – 25 patients (25%), migraine – 9 patients (9%), and dizziness (of unknown etiology) – 8 patients (8%). The majority of patients (59%) were aged 51–75, 23% aged 26–50, 14% aged over 76 and 4% aged under 25. This study revealed that dizziness was observed the most commonly – in 69% of patients. 74% of patients with dizziness were hospitalized because of a vascular incident in the brain (28/34 – 82% of stroke patients and 23/25 – 92% of patients with TIA). As regards the character of dizziness episodes in this group: the majority of patients after stroke (21/34 – 64% of stroke patients) experienced permanent dizziness, while in TIA patients the dizziness was of paroxysmal (11/25 – 44%) and permanent (9/25 – 36%) character. Several typical elements of examination were observed in patients with vascular incidents in the brain: a positive Romberg Test (neurological examination) and Sensitized Romberg Test (physiotherapeutic examination) – 71% (21/34 after stroke, 21/25 patients with TIA) and a positive result of Sensory Integration Test – 63% (22/34 after stroke and 18/25 with TIA). The illusion of rotational movement characteristic of vertigo was found in 23% of all patients. They were mostly diagnosed with BPPV (benign paroxysmal positional vertigo), BPPV with hydrocephalus and in case of two patients – Meniere's disease (2%). The Romberg Test, Sensitized Romberg Test and Sensory Integration Test were positive in 30% of patients with vertigo.

Discussion

Dizziness was the presenting complaint of almost every twentieth patient admitted to our neurological ward during the two-year observation period. The results confirm that vertigo is a common symptom that a neurologist has to deal with on a daily basis on duty. Moreover, in most cases, the assessment allowed for a proper diagnosis and the determination of the causes of those ailments.

The majority of studies concerning the epidemiology of different kinds of dizziness were conducted in EDs (emergency departments). However, ED diagnoses are mostly unconfirmed, which might bias the results [11], e.g. wrong diagnoses might be made in cases of cerebrovascular causes of dizziness. According to Kerber et al. [12] even 35% of such patients might be misdiagnosed. Differences between diagnoses are shown in **Figure 1** which confirms the importance of detailed assessment in neurology departments – for instance, only 17 cases out of 34 strokes (diagnosed in neurology units) were diagnosed in EDs or 4 TIA cases in EDs vs. 25 final diagnosed cases. Misdiagnosis may be very dangerous and have negative consequences for patients, e.g. the lack of adequate acute treatment.

Some patients find it difficult to describe the specific type of vertigo/dizziness, so it is often complicated to distinguish between these types in acute patient assessment, for example in ED.

A study by Kevin et al. [18] showed that patients with a diagnosis of stroke or TIA reported: dizziness in 23 cases, vertigo in 18 cases, imbalance in 11 cases, and more than one of those problems in 1 case (of all 53 cases). Our study revealed that almost 3/4 of patients with dizziness were hospitalized because of a vascular incident and they declared different characters of dizziness.

Some dizziness cases are specific for neurology specialists. However, according to Nowaczewska [13] they were still problematic in terms of diagnosis, which was also reflected in the results of this study as unknown-etiology cases of dizziness.

Sandlund et al. [14] pointed out that a better management algorithm could improve the quality of care for dizziness patients.

The authors found only one similar study published by Weisshaar et al. [19] in October 2019. A total of 11% of patients of a Norwegian hospital had dizziness as the primary symptom in a 1-year assessment period, in contrast to 4.6% found in our study. However, some groups were excluded from our study, e.g. people with communication problems.

According to the latest research during the SARS-Cov-2 pandemic the problem of dizziness evaluation should also be mentioned. A study by Mao et al. [15] demonstrated that dizziness was one of the most common neurological manifestations of COVID-19.

This research has several limitations – the study sample should be larger. However, it was a pilot study, so more data will be collected. Moreover, study design could be planned with interviewers blinded to case/control status, so as any information on the outcome cannot influence the collection of information – to reduce possible bias. Furthermore, the authors might consider a more detailed diagnosis, e.g. the exact location of the lesion in stroke patients – the authors focused on the cerebrovascular causes of dizziness in general in the pilot study.

Conclusion

1. Dizziness is a common symptom that requires consultation by a neurologist in the emergency room.
2. A variety of diagnoses were demonstrated in patients hospitalized in the neurological department in whom the occurrence of dizziness was the presenting complaint.
3. The role of a physiotherapist in the diagnostic process and treatment planning in patients with dizziness of various origins was indicated.

Clinical implications/ future directions

Future research is needed to be performed in a larger group to prepare the effective assessment procedure in dizziness cases including physiotherapy evaluation. It may provide a framework for dizziness management and give directions to the diagnoses of dizziness and treatment options to be used in neurology departments.

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Conflict of interest statement
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REVIEW PAPER

JMS Journal of Medical Science

Hypothetical Immunological and Immunogenetic Model of Heterogenous Effects of BCG Vaccination in SARS-CoV-2 Infections: BCG-induced Trained and Heterologous Immunity

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ABSTRACT

Though SARS-CoV-2 infections are yet to be completely characterised in a host-pathogen interaction context, some of the mechanisms governing the interaction between the novel betacoronavirus and the human host, have been brought to light in satisfactory detail. Among the emerging evidence, postulates regarding potential benefits of innate immune memory and heterologous immunity have been put under discussion. Innate immune memory entails epigenetic reprogramming of innate immune cells caused by vaccination or infections, whereas heterologous immunity denotes cross-reactivity of T cells with unrelated epitopes and bystander CD8+ activation. Familiarization of the host immune system with a certain pathogen, educates monocytes, macrophages and other innate cells into phenotypes competent for combating unrelated pathogens. Indeed, the resolution at which non-specific innate immune memory occurs, is predominant at the level of enhanced cytokine secretion as a result of epigenetic alterations. One vaccine whose non-specific effects have been documented and harnessed in treating infections, cancer and autoimmunity, is the Bacillus Calmette–Guérin (BCG) vaccine currently used for immunization against pulmonary tuberculosis (TB). The BCG vaccine induces a diverse cytokine secretion profile in immunized subjects, which in turn may stimulate epigenetic changes mediated by immunoreceptor signalling. Herein, we provide a concise summarization of previous findings regarding the effects of the BCG vaccine on innate immune memory and heterolo-

gous immunity, supplemented with clinical evidence of the non-specific effects of this vaccine on non-mycobacterial infections, cancer and autoimmunity. This interpretative synthesis aims at providing a plausible immunological and immunogenetic model by which BCG vaccination may, in fact, be beneficial for the current efforts in combating COVID-19.

Background

The COVID-19 pandemic is a rapidly evolving situation, with novel information emerging from the academic ether on a daily basis. Though the immunobiological details of SARS-CoV-2 infections continue to be uncovered in a rapid rate, a modest number of mechanisms governing the interaction between the novel betacoronavirus, have been brought to light at a satisfactory level of detail. Among the evidence that has emerged since the onset of the pandemic, postulates regarding the potential benefit of innate immune memory and heterologous immunity have been put forth and continue to be discussed. Innate immune memory entails epigenetic reprogramming of innate immune cells caused by vaccination, or viral and bacterial infections, whereas heterologous immunity colloquially denotes cross-reactivity of T cells with unrelated epitopes, along with bystander $CD8^+$ activation [1]–[11]. On the innate level, familiarization of the host immune system with a certain pathogen, may educate monocytes, macrophages and other innate cells into becoming more competent in combating non-related bacterial or viral pathogens [10], [12]. Interestingly, however, T cell cross reactivity likely stems from host genetic factors rather than pathogen-induced epigenetic reprogramming [13]–[19]. Indeed, the resolution at which bacteria, viruses and vaccines confer non-specific effects that lead to innate immune memory, is likely at the level of enhanced cytokine secretion as a result of epigenetic alterations [5], [9], [10], [20]–[27]. One vaccine whose non-specific effects have been documented and harnessed in treating infections, cancer and autoimmunity, is the Bacillus Calmette–Guérin (BCG) vaccine currently used for immunization against pulmonary tuberculosis (TB). The BCG vaccine induces a diverse cytokine secretion profile in immunized subjects, which in turn may stimulate potentially beneficial epigenetic changes mediated by immunoreceptor signalling [8], [28]–[31]. Additionally, the phenomenon of heterologous immunity has not only been observed in cases of BCG vaccination. The influenza vaccine may confer varying degrees of protection against severe forms of COVID-19 disease and presumably SARS-CoV-2 infection. This is reflected in studies where patients receiving the influenza vaccine within 120 days of a positive diagnosis were at a reduced risk of post-COVID-19 complications, further coupled with a decreased rate of COVID-19 positive cases among vaccinated populations [32]–[34]. However, much like in the case of BCG vaccination, more work is required to derive a definitive conclusion. Unsurprisingly, in a recently published preprint by Föhse et al. it was reported that the COVID-19 BNT162b2 mRNA vaccine likely induces complex innate immune system reprogramming at the level of cytokine regulation, offering protection against unrelated bacterial, fungal and viral stimuli [35].

A correlation between reduced COVID-19 morbidity and universal BCG vaccination has been implied since the early stages of the pandemic, though the immunobiological background and potential clinical significance of this remains to be substantiated [36]. Indeed, BCG vaccination leads to cellular memory at the level of both cytokines and cytokine-related transcription factors, some of which have been identified as potential targets of SARS-CoV-2 in order for the virus to establish immunosuppression [37]–[40]. The importance of this is reflected in the fact that SARS-CoV-2 dampens the adaptive immune response by acting directly on the transcriptional machinery of innate immune cells. Considering that the BCG vaccine leads to epigenetic changes that may be beneficial in preventing SARS-CoV-2-mediated immunosuppression or dissemination, this issue must be addressed in a methodical way that draws back to basic immunobiology, rather than mere statistical epidemiology. Herein, we provide a concise summarization of previous findings regarding the effects of the BCG vaccine

on innate immune memory and heterologous immunity, supplemented with clinical evidence of the non-specific effects of this vaccine on non-mycobacterial infections, cancer and autoimmunity. This interpretative synthesis aims at providing a plausible and unbiased immunological and immunogenetic model by which BCG vaccination may, in fact, be beneficial for the current efforts in combating COVID-19.

Cellular Entry of SARS-CoV-2

SARS-CoV-2 infections share similarities with the Middle East Respiratory Syndrome (MERS)-CoV and Severe Acute Respiratory Syndrome (SARS)-CoV in their mode of interaction with the human host. There is significant receptor binding domain (RBD) similarity between SARS-CoV and SARS-CoV-2 found on the spike

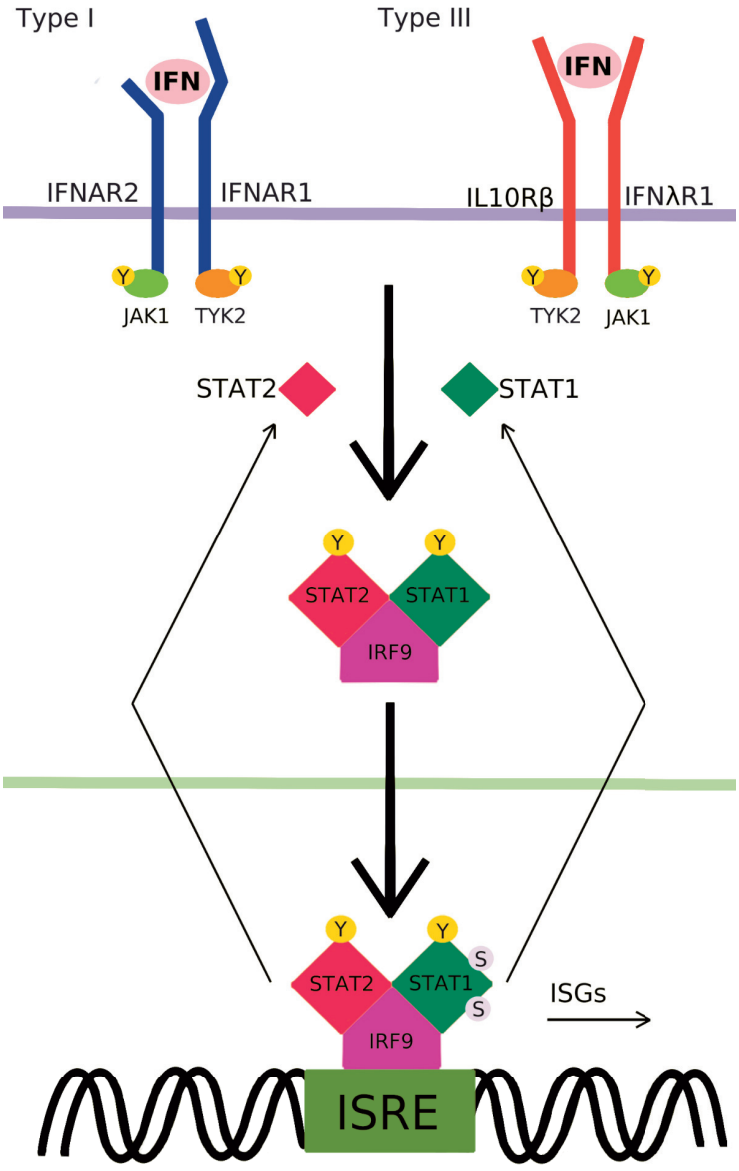


Figure 1. Type I and type III interferon responses are pivotal in the human innate antiviral response. Canonically, type I IFN signalling eventuates in the activation of the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) 2 proteins, whereas type III IFN responses recruits STAT1. Interferon regulatory factor (IRF) 9, particularly relevant in the antiviral response, associates with the JAK-STAT dimer, thereby creating the IRF9 transcription factor. IRF9 transcription factor is translocated into the nucleus, upon which it binds to the interferon stimulated response element (ISRE) located upstream of the interferon stimulated genes (ISG)

(S) protein of both viruses [41], [42]. SARS-CoV and SARS-CoV-2 infect cells expressing angiotensin converting enzyme 2 (ACE2), located in the lungs, the gastrointestinal tract, the renal tract and the heart [41], [43]–[45]. SARS-CoV-2, however, has overall higher binding affinity for ACE2 than SARS-CoV, and this is particularly pronounced for several clinically-relevant variants [39], [46], [47]. Once the S protein is bound to ACE2, ADAM metallopeptidase domain 17 (ADAM 17) and other sheddases cleave the extracellular domain as a method of preventing cellular entry. ADAM 17 further processes the membrane form of the interleukin (IL)-6 receptor (IL-6R)-α into a soluble form that will confer activation of signal transducer and activator of transcription 3 (STAT3) in non-immune cells, under the mediation of gp130. STAT3, in turn, activates the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway, leading to potentially detrimental inflammatory responses [48].

It is possible that SARS-CoV-2, like MERS-CoV and SARS-CoV, binds to non-ACE2 receptors through carbohydrate binding, specifically various lectins and different glycoconjugates of different bacterial strains that comprise the lung microbiota [49]. Such findings offer clues for the immunosuppressive capabilities of SARS-CoV-2, particularly when discussing the notion that the viral RBD domain binds to C-type lectins such as CD209/DC-SIGN and CD209/L-SIGN, which would presumably allow the virus to infect innate and adaptive immune cells [38], [50], [51].

Immune Response to SARS-CoV-2

Innate Immune Response

Upon entry of the virus into the cell, cytosolic recognition of RNA viruses by innate immune cells occurs at the interface between the viral RNA or replication intermediates and the innate cytosolic RNA sensor, toll-like receptor (TLR) 3 and TLR7 and the cytosolic dsRNA sensor retinoic acid-inducible gene (RIG) I/ melanoma differentiation-associated protein (MDA) 5 [52]. Production of type I interferon (IFN) is triggered when viral pathogen-associated molecular patterns (PAMPs) are recognized by these receptors, activating NF-κB and interleukin regulatory factor (IRF) 3, which are then translocated into the nucleus to initiate transcription of pro-inflammatory cytokine genes, including IFN type I [53]. Successful secretion of IFN in the cytosol triggers the Janus kinase (JAK) - signal transducer and activator of transcription (STAT) 1 pathway, through the interferon-α/β receptor (IFNAR) (**Figure 1**) [53]. Although the role of DCs, and particularly resident respiratory DCs (rDCs) in SARS-CoV-2 infections warrants further research, the clinical presentation of COVID-19 is likely in part owed to altered DC function, thereby preventing their migration to the mediastinal and cervical lymph nodes in order to prime virus-specific T cells [54], [55]. *Per contra*, impaired rDC migration has been correlated with age, thereby offering another plausible explanation, or at least a relevant factor, to the discussion COVID-19 risk groups [55]. Since SARS-CoV-2 is particularly efficient at avoiding IFN-mediated innate immunity, this leads to massive immunopathology or extensive viral replication in the lungs and the respiratory tract, thereby often warranting a need for patient hospitalization in the confines of intensive care.

Adaptive Immunity in COVID-19

The issue of SARS-CoV-2 adaptive immunity, specifically protection longevity and its correlation to emerging viral variants, continues to be investigated and awaits definitive conclusions. Though certain studies have reported antibody longevity supported by long-lived bone marrow plasma cells (BMPCs), some evidence suggests that the neutralizing capability of these antibodies for SARS-Cov-2 variants is rendered unsatisfactory over time, at least for the S protein [56]–[58]. This is supported by studies reporting re-infections with genomically distinct SARS-Cov-2 variants [59], [60].

Secretion of cytokines and antigen presentation by antigen presenting cells (APCs) helps prime and direct the adaptive immune response to infections [61]. The Th1 immune response is the key player in response to viral agents, and was shown to be particularly relevant for resolving infections with SARS-CoV and MERS-CoV and, unsurprisingly, SARS-COV-2 [52], [62]. In the case of SARS-CoV infections, the specificity of B and T cell epitopes were mapped to the M, N, E and S viral proteins [63]. For SARS-Cov-2, however, these epitopes have thus far been mapped

to non-structural proteins (nsps), particularly nsp3, nsp5, the nucleocapsid (N) protein, the S protein and the open reading frame (ORF) 3a [64]. Interestingly, an IgM response targeting nsp3 and nsp5 have been correlated with a better prognosis of COVID-19, whereupon an IgG targeting S, N and ORF3a are associated with mortality and increased severity [64]. Namely, the serum of COVID-19 patients shows moderate cross-reactivity with SARS-CoV and no reactivity for other coronaviruses [65]. In terms of seroconversion, Zhao et al. found that, among 173 patients whose samples were analysed, seroconversion time for Ab, IgM and IgG was 93.1% (161/173), 82.7% (143/173) and 64.7% (112/173) respectively . Specifically, antibody presence was determined to be < 40%; however, after day 15, this significantly changed to 100.0%, 94.3% and 79.8% for Ab, IgM and IgG respectively, and relatively similar results were obtained in other studies [66]–[69]. Interestingly, long lasting IgG and neutralizing antibodies have been reported even 2 years upon initial diagnosis with SARS-CoV, and there is encouraging evidence that the same may be true for SARS-CoV-2 [70]. As evidence continues to emerge, it will be interesting to see whether the aforementioned long-lasting neutralizing antibodies following SARS-Cov-2 infection will carry sufficiently broad specificity for emerging variants in terms of the S protein and other immunogenic viral proteins.

Increase in serum Th2 cytokines were detected SARS-CoV, along with a higher frequency of polyfunctional $CD4^+$ T cells secreting tumour necrosis factor (TNF) α, IFN-γ and IL-2 in severely ill SARS-CoV patients; an overall increase in serum Th2 cytokines were present in patients that faced a fatal outcome [71]. However, it should be noted that $CD8^+$ cells dominate over $CD4^+$ in SARS-CoV, and strongly neutralizing Abs are present in convalescent patients [71]. As one may infer from the herein presented immunological data, severe lung immunopathology occurs at the delicate interface between the Th1 and Th2 immune response, yet commences at the level of innate immunity. Reducing IFN-mediated infection control allows SARS-CoV-2 to evade immune defences and delay the onset of adaptive immunity, which later results in rampant inflammation that damages the protective epithelial alveolar tissue comprised of ACE2-expressing type II alveolar cells [72], thus leaving the pulmonary tissue vulnerable to development of bacterial pneumonia [65]. Patients void of certain medical conditions generally fare better than those who are immunocompromised or who have previously been diagnosed with a condition that may be detrimental for competently combating viral infections [43].

Immune Evasion Tactics of SARS-CoV-2

SARS-CoV dampens the JAK-STAT pathway, which seems to be mechanism likely utilized by SARS-CoV-2 for immune evasion [52], [53]. This results in delayed onset of the INF-mediated anti-viral response by way of underexpression of genes containing interferon stimulated response element (ISRE), which has thus far been supported by *in vivo* and *ex vivo* studies on SARS-CoV and MERS-CoV [73]–[75]. SARS-CoV successfully interferes with induction with type I IFN by interfering with downstream signalling of cytosolic RNA sensors, through ubiquitination and subsequent degradation of their adaptor molecules, or by inhibiting the translocation of IRF3 into the nucleus by way of non-structural proteins PLpro and ORF3b [53], [76], [77]. Expressed both by MERS-CoV and SARS-CoV, PLpro has also been shown to inhibit dissociation of NF-κB from I-κB, which in turn inhibits the proper functioning NF- κB transcription factor [78]. By reducing the host's ability to control the infection, SARS-CoV-2 is able to freely replicate within the infected cell, and the mechanisms by which these evasion tactics eventuate leads to extensive inflammatory immunopathology. The reduced IFN-mediated viral control paves the way for viremia, as suppression of type I and III interferons leads to insufficient expression of interferon stimulated (ISG) genes [43]. These findings are in favour of the hypothesized pathogenesis discussed by Lin et al., who made the observation that acute respiratory distress syndrome (ARDS) is initiated somewhere around day 8 of disease onset, likely due to the overwhelming increase in pro-inflammatory cytokines, neutrophils and other immune cells which cause detrimental inflammatory damage to the host when excessively recruited [43]. Further supporting this hypothesis are data from

138 hospitalized COVID-19, where an increase in neutrophils, proinflammatory cytokines, D-Dimer and lymphopenia were detected in severely ill or deceased patients, contrasted with those who successfully recovered [79].

Dysregulation of functional T cells is particularly pronounced in SARS-CoV and likely SARS-CoV-2 infections, leading to overexpression of the programmed cell-death protein (PD)-1, T cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) and T cell immunoreceptor with Ig and ITIM domains (TIGIT), as a consequence of excess production of IL-6, IL-6 and TNF-α [80]–[83]. Inducing overexpression of the aforementioned proteins is a sensical approach for SARS-CoV-2 to take. After all, even when minutely expressed on the surface of T cells, PD-1 negatively regulates T cell activity and sees elevated expression in exhausted T cells [84]. Furthermore, hierarchical T cell loss, along with T cell suppression and dysfunction are mediated by high expression levels of TIM-3 via impedance of cytokine production, particularly TNF and IFN-γ [85]. Expressed as a coinhibitory receptor on natural killer (NK) cells, memory T cells, follicular Th cells, and on a subset of regulatory T cells (Tregs), TIGIT engagement leads to inhibition of Th1 and Th17 cell response [86]–[90]. TIGIT ligation has been shown to directly suppress T cell proliferation and cytokine production of CD^{4+} T cells. Furthermore, TIGIT may indirectly inhibit T cell response through CD155 in DCs, leading to the production of the immunosuppressive cytokine IL-10 [90]. Though substantial work stands in the way of more comprehensive understanding, it is clear that SARS-CoV-2 likely utilizes similar immune evasion mechanisms as MERS-CoV and SARS-CoV in order to circumvent the human immune system.

The Bacillus Calmette-Guérin Vaccine

The Adaptive and Innate Immune Response to BCG Immunization

Despite it being the only approved vaccine for TB, the protection it offers is quite heterogenous in adults and adolescents (0 – 80%) [91]. This includes heterogenous efficacy in the context of its initial purpose, which is prevention of disseminated TB, tuberculous meningitis and severe forms of TB in children, where factors such as geographical location influence vaccine efficacy, though neonatal and postnatal administration of the vaccine offers decent protection against paediatric cases of disseminated TB and meningitis (60-80%) [92]–[94]. In spite of this, the current consensus is that there is an urgent need for a novel TB vaccine [95]. Even the induced cytokine profiles vary across populations. Evidence of the benefits of re-vaccination is relatively scarce and inconclusive, although it has been postulated that it does induce cellular and humoral immunity to an unclear extent [11], [91], [96], [97]. Administration routes may also play a role in the varying efficacy and limited protection [93]. Though most studies regarding discrepancies between BCG administration routes and their effects on efficacy stem from animal models, certain human studies have shed light on how different strains elicit distinct immune responses [98], [99]. This is reflected in differences between efficacy in the induction of specific IgG and IgA against various mycobacterial components such as lipoarabinomannan (LAM). The intranasal administration of BCG induces increased production of specific and non-specific IgG and IgA through IL-17 in mice [100], [101]. Studies on Rhesus monkeys and guinea pigs found that aerosol BCG administration increased protection to virulent *M. tuberculosis* challenge, although antibody production was never measured in human aerosol BCG studies [102]. Interestingly, an NHP-based study on intravenous (IV) BCG injections [103], revealed strikingly improved protection and precipitating antibodies post-vaccination, namely IgG, IgM and IgA. Currently the specific protective implications of these findings warrant further research, however such striking findings are native to IV BCG injections alone [91], [104]. Efficacy of the BCG vaccine presumably varies accordingly to the virulence of the BCG strain, however there is no sufficient data that clearly elucidates the true depth of the immunogenicity of different strains and how it confers protective immunity and non-specific effects [91], [105], [106].

Another layer of complexity is added to the topic of BCG strains by the presence of environmental mycobacteria that humans are exposed to in varying degrees across different geographies [107]–[110]. Limited data is available on the

antigens related to environmental mycobacteria, thus making the differentiation between different T-cell responses and various environmental mycobacteria, increasingly difficult, particularly in the context of BCG [93]. However, different B cell epitopes for different BCG strains were proposed as the plausible cause of heterogenous efficacy [111]. It should be noted that the overall topic of the humoral response to BCG vaccination has been modestly investigated. Regardless, the scarcity of comprehensive studies on this particular topic does not rule out the potentially significant effects that BCG vaccination has on humoral immunity.

Innate immune memory is not a novel concept, and has been previously explored to varying degrees of success in the context of BCG and other pathogens. Immunological memory of innate immune cells, though in defiance of the dogmatic classification of the innate immune system as void of permanent memory, has been compellingly challenged in recent years. It is known that exposure to PAMPs leads to improved innate immune response to bacterial and viral infections, though the underlying mechanisms behind this are poorly understood [6], [7]. Interactions between cell surface receptors of innate immune cells and their agonists, appear to be the driving force of these long-lived cellular memory. Despite there not being a comprehensive map displaying the ways in which BCG confers innate immune memory, numerous studies have validated the assumptions that BCG may effectively be used in non-mycobacterial infections for therapeutic purposes. Interestingly, BCG-induced training of the innate immune system seems to be completely independent of B and T cells.

Upon administration of the vaccine via intradermal injection, a pro-inflammatory response is elicited at the injection site, which includes IL-1β, TNFα, monocyte chemoattractant protein-1 (MCP-1/CCL2), and IL-8, the source of which are local innate immune cells [112], [113]. Stimulation of monocytes/macrophages with these and other cytokines have been correlated with trained immunity. Interestingly, BCG-enhanced IL-1β production has been strongly correlated with human trained immunity that offers protection against the Yellow fever Virus (YFV) [114], [115]. Innate immune cells migrate to the injection site around day 9 post-vaccination. Adult humans that have been BCG vaccinated for the first time have lingering BCG at the injection site for approximately 4 weeks, eventuating in a cellular infiltrate comprised of mostly of $CD15^+$ neutrophils, although $CD3^+$ lymphocytes and $CD14^+$ monocytes may also be found [93], [106], [116]. Migration of APCs carrying live mycobacteria or mycobacterial antigens to proximal lymph nodes, under the mediation of type I polarizing cytokines and IFN-γ, results in education of naïve T-cells into $CD4^+$ and CD^{8+} cells [107], [117], [118]. Presence of IFN-γ further propagates antimycobacterial activity of macrophages and mediates enhanced antibody production by plasma cells [119], [120]. A pool of mycobacteria-specific CD^{8+} cells that secrete IFN-γ and express granzymes and perforins, are detectable in peripheral blood up to 10 weeks post-vaccination in human newborns [121], [122]. Furthermore, large amounts of TNF-α, IL-2 and IFN-γ are produced by Th1 $CD4^+$ cells, which were also detectable in *ex vivo* studies investigating BCG-immunized newborns [123]–[125]. Enhancement of the T-cell response to BCG administration is conferred by neutrophils ingesting live BCG [126], [127]. Although macrophages, NK cells and monocytes have been given the most attention in studies regarding innate immune memory, DCs may also garner phenotypic changes that favour long-lived immunological memory [128], [129]. 4-8 weeks upon BCG immunization, a long-lived B cell response is induced, eventuating in an increase in secretion of IgG [119], [130] .

A Model of Innate Immune Memory

Epigenetic Modifications of Cellular Memory and Response Genes in Innate Phagocytic Cells: TLR Signalling

Exposure to the mannose-capped lipoarabinomannan (ManLAM) found on the cell walls of *M. tuberculosis*, BCG and other mycobacteria, promote IL-8 secretion specifically by macrophages, which further stimulates recruitment and activation of neutrophils [27], [131]–[134]. However, other BCG molecular patterns may also be involved. Stimulated neutrophils prime macrophages into phenotypes that confer protection against a wide variety of pathogens, and such phenotypes demonstrate longevity both upon BCG vaccination and stimulation by non-mycobacterial PAMPs [112], [113], [134]. The root of this longevity may

indeed be found in the epigenetic reprograming of innate immune cells, as such phenotypes evidently extend towards myeloid progenitor cells, with TLR signalling being heavily implicated in the process [24].

Generally, TLR-associated macrophage inflammatory genes may be differentiated into primary response genes (PRGs) and secondary response genes (SRGs), with PRGs being induced within approximately one hour upon stimulation [24]. TLR ligation confers permissive chromatin regions as a result of histone H3 lysine residue 4 trimethylation (H3K4me3) and H3 acetylation (H3A) (**Figure 2**) [24]. Such epigenetic modifications lead to transcriptionally engaged RNA polymerase II (RNA pol. II) being bound to the promoter proximal regions of stimulus-responsive PRG, even after stimulus-induced signalling. Under homeostatic conditions, certain PRGs have higher basal transcriptional activity even in the absence of stimulus due to higher levels of H3K4me3 within their transcription start sites (TSS) [24]. Of course, TLR signalling enhances the transcriptional activity of such genes. These basal epigenetic modifications have been heavily correlated with the binding of the specificity protein (Sp1) transcription factor to GC-abundant CpG elements found within the PRG promoters [23], [113].

An emphasis to extend of this epigenetic programming, are findings pertaining to the presence of protective BCG-trained monocytes 3 months following vaccination, and the underlying mechanism was associated with H3K4me3 and H3A on promoters associated with PRG [22]. BCG vaccination significantly increases trimethylation of PRG promoters by way of TLR4 and IFN-γ-mediated signalling in macrophages, though other TLRs are very likely involved at least on a monocyte differentiation level [135]. Considering that monocytes express each type of TLR, BCG vaccination could induce their epigenetic reprogramming via TLR signalling, thereby causing their differentiation into phenotypes of trained immunity [136]. These phenotypes may show increased potency for the clearance of viral infections, as the aforementioned cells are the first ones to encounter viral and bacterial pathogens.

Interferons and Epigenetic Modification of Interferon-stimulated Genes

BCG vaccination induces an IFN-γ response through stimulation with numerous mycobacterial antigens [28], [93]. Though BCG-induced innate immune cell memory phenotypes are increasingly studied in the context of protection against bacteria and fungi, it may render the innate immune system better equipped for viral infections with

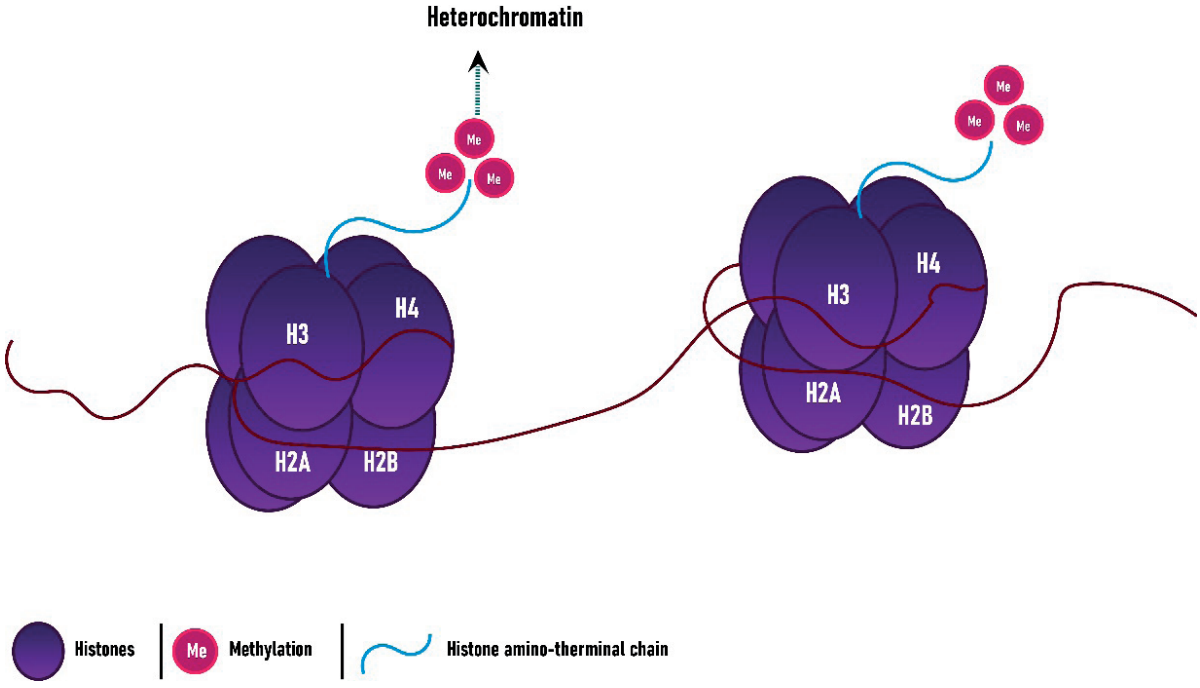


Figure 2. Histone H3 trimethylation at lysine residue 4 at promoter-associated GC-abundant CpG elements, is an epigenetic modification associated with trained immunity

RNA viruses such as SARS-CoV-2. Epigenetic modifications committed to cellular memory upon IFN exposure open some interesting questions with regards to SARS-CoV-2 immune evasion tactics, the answers to which are gradually emerging. BCG vaccination leads to increased production of IFNs, such as IFN-β and IFN-γ, therefore creating optimal conditions for epigenetic modifications [25], [26], [137], [138]. Whether this holds any merit for COVID-19 prophylaxis or treatment remains to be determined.

The relevance of type I and II IFN in antiviral response has been fastidiously substantiated, thereby making these IFN classes integral in the discussion of SARS-CoV-2 immunopathology [26]. IFN stimulation of macrophages eventuates in the creation of chromatin marks for transcriptional memory via histone trimethylation of histone H3.3 and H3K36me3 [113]. The ISGs that take part in macrophage cellular memory have been thoroughly studied, though distinct sets of genes of other innate immune cells may undergo similar modification when adequately stimulated. Interestingly, IFN memory evidently depends on functional STAT1, whereas STAT3 appears to be redundant for induction of IFN memory phenotypes [26]. Contextually to innate immune memory, ISGs may be divided into refractory (108), memory (66) and non-memory (251) ISGs, and this was elegantly demonstrated by Kamada and others in their work on IFN-induced macrophage memory [26]. Marks of permissive chromatin are most prominent in the memory-associated genes, with increased RNA pol. II binding status in contrast to refractory and non-memory ISGs [139]. Though BCG vaccination induces IFN-γ, thereby conferring epigenetic modifications of ISGs, it is likely that this represents only a component of innate immune memory, rather than the underlying mechanism.

BCG-induced Epigenetic Modifications Through NOD2 Signalling

BCG-induced trained immunity likely depends on a large number of host-specific, environmental and vaccine-related factors, with modest progress in identifying PAMPs that promote epigenetic modifications (**Figure 3**). Progress made in recent years, however, points to host receptors playing a particularly relevant role in acquiring phenotypic traits of trained immunity, coupled with a miniscule number of identified antigens. For instance, muramyl dipeptide (MDP) found in mycobacteria, including BCG, has been shown to confer viral protection in a nucleotide-binding oligomerization domain-containing protein 2 (NOD2) and IFN-β-mediated fashion [37], [140]. MDP treatment of cell lines before or after infection induces NF-κB and mitogen-activated protein kinase (MAPK) cascades, with potential relevance with immunosuppressive infections with pathogens such as SARS-CoV-2 [139], [141]. In fact, *in vitro* pre-treatment of fibroblasts with MDP leads to human cytomegalovirus (HCMV) suppression upon NOD2 ligation; an outcome that is IFN-β dependent and suggestive of the relevance of NOD2 in viral infections [142], [143]. Considering that both DNA and RNA viruses and their corresponding PAMPs are NOD2 agonists, modifications of genes associated with NOD2 signalling may be particularly relevant for the innate immune response to SARS-CoV-2, assuming *a priori* acquisition of trained phenotypes [140]. Furthermore, basal expression levels of NOD2 are higher in macrophages and monocytes in contrast to fibroblast, therefore NOD2 mediated signalling is likely more pronounced in these cells. Of course, this increased potency may translate to increase efficiency with regards to trained immunity.

NOD2 signalling leads to IκB kinase complex (IKK) activation in order to degrade the inhibitory IκBα protein. Synergic IFN-γ and MDP signalling leads to increased IKK activation, thereby significantly reducing IκBα levels in a STAT1 independent fashion [144]. Though understanding of IKK regulation is incomplete, it is known that TNF-α negatively regulates IKK activity by C-terminus phosphorylation of the IKKβ subunit [28]. Taking this into consideration, it is not difficult to infer that BCG-induced TNF-α may render the BCG vaccine inadequate for therapeutic purposes in COVID-19. A possible way out of this conundrum may lie in the heterogenous BCG-induced cytokine profiles across different populations, which opens the possibility of tailoring different BCG strains in accordance to the populational response [113]. Evidence supporting this suggestion may be extrapolated from the work of Kleinnijenhuis and others, where BCG-induced trained immunity of human monocytes was achieved in a NOD2 and Rip2 dependent manner [115], [145],

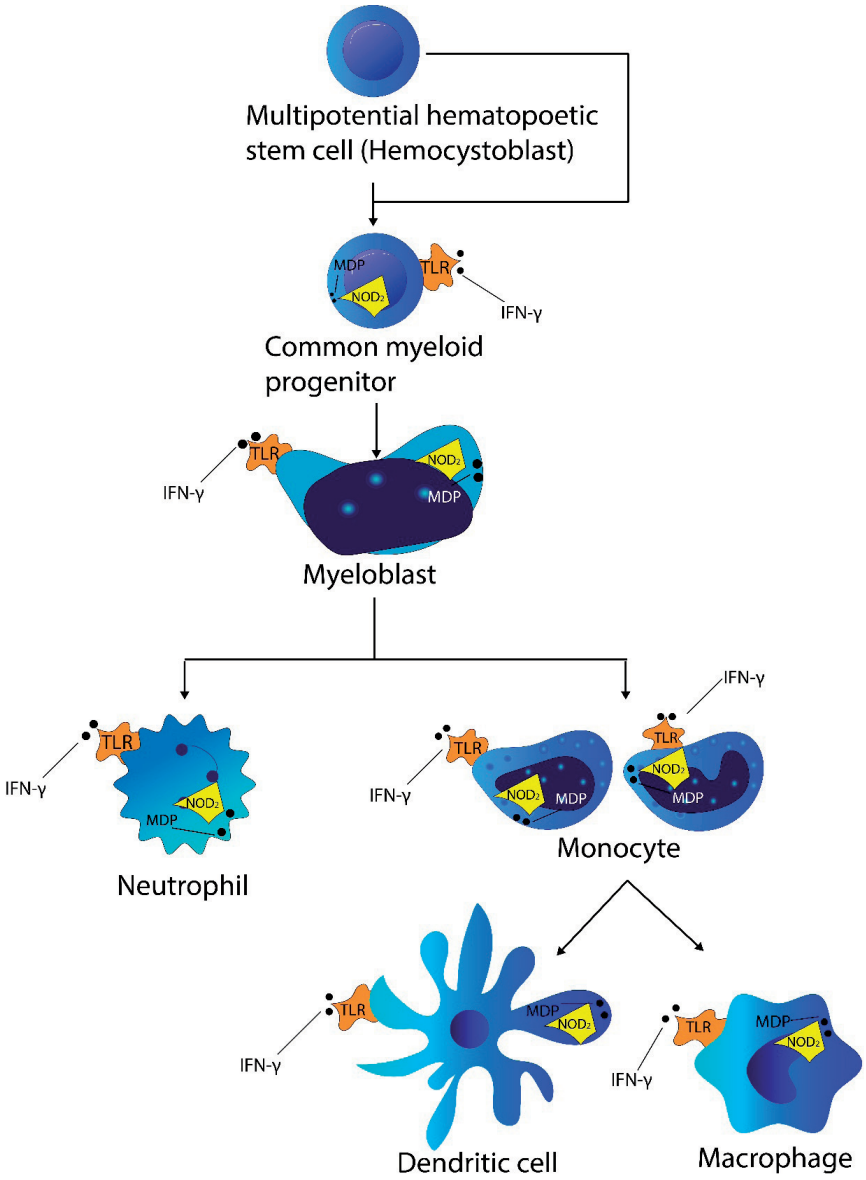


Figure 3. Different cells of the innate immune system can undergo cytokine/antigen-stimulated epigenetic changes that may induced trained immunity. Common myeloid progenitor cells may also be stimulated, thereby differentiating into trained phenotypes. Abbreviations: MDP – muramyl dipeptide, IFN-γ – Interferon Gamma, TLR – Toll Like Receptor, NOD2 – Nucleotide-binding oligomerization domain-containing protein 2

[146]. Strikingly, their work demonstrated redundancy of TLR2 and TLR4 in this process, though it is likely that other mechanisms take part in this process that were simply not considered in the work. Considering that SARS-CoV-2 likely blocks IκB dissociation from NF-κB, it would be interesting to see whether NOD2-dependent trained immunity entails phenotypes that are more resilient to this tactic. Redundancy of TLR2 and TLR4 does not exclude the roll of TLRs in trained immunity, but rather emphasize the complexity and heterogeny of the mechanisms behind it.

A Model of Heterologous Adaptive Immunity for SARS-CoV-2 Infections

Vaccines were initially considered to eventuate in immune responses precisely tailored towards the pathogen-associated antigen contained within the vaccine. Canonically, once the phagocytic cells engulf an antigen/pathogen, they migrate to proximal lymph nodes and present pathogen-associated peptides (epitopes) to naïve T cells via the type I/II major histocompatibility complex (MHC I/II). In turn, this leads to T and B cell priming, followed by their clonal expansion. The

traditional interpretation of adaptive immune memory infers that educated lymphocytes are specific only for the epitopes presented by way of MHC molecules. Whilst this specificity, indeed, is predominantly present in the human immune system, a closer inspection of T cell reactivity reveals evidence of heterogenicity, colloquially termed "heterologous immunity". The "off target" vaccine effects that give rise to heterologous immunity lead improved responses to unrelated pathogens and immunological tolerance in autoimmune conditions, though negative effects have also been documented [145], [147]–[151]. Specific mechanisms behind heterologous immunity are poorly understood, however epigenetic programming, cross-reactivity between epitopes and changes in metabolic profiles of lymphocytes, likely play major roles [152]–[155].

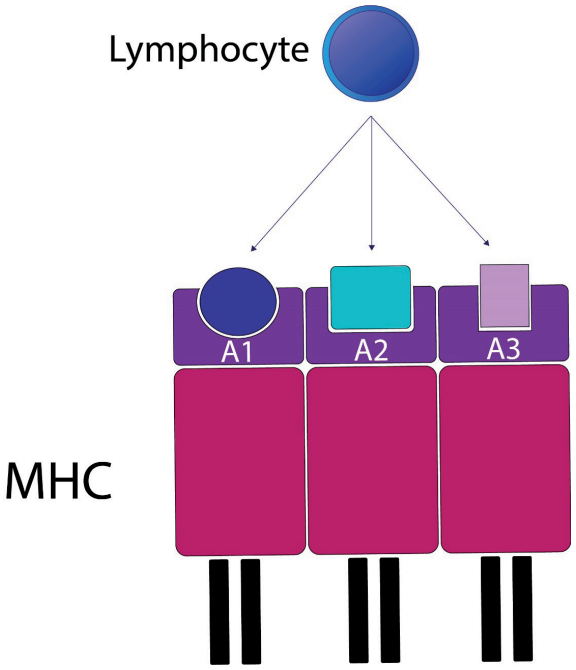


Figure 4. Cross-reactive lymphocytes can respond to different antigen determinants presented on the MHC grooves. Though T cells are most prominently known for cross-reactivity, B cells may also be cross reactive [158]. Abbreviations: MHC – Major Histocompatibility Complex

Immunological Cross-reactivity of T Cells

APCs present pathogen-associated epitopes by way of MHC I and II to $CD8^+$ and $CD4^+$ T cells respectively, in the form of short amino acid sequences (MHC I: 8-11, MHC II: 13-17). Hitherto proposed to depend on the presentation of such conserved linear sequences by clonal selection theory, T cell reactivity evidently extends towards completely unrelated antigenic determinants presented from the MHC antigen-binding groove [152]–[155] (**Figure 4**). Considering that the amino acid sequences that garner heterologous T cell reactivity are modestly homologous, regular immunological cross-reactivity may be possible not only for unrelated infections, but detrimental in the context of autoimmunity [156], [157]. The root of this heterology is poorly understood, though several plausible mechanisms have been suggested, all of which may, singularly or synergistically, share responsibility for this phenomenon. Though heterologous immunity has thus far been documented in the context of viral infections, BCG vaccination may indirectly lead to heterology through induction of cytokine secretion.

Phenotypic alterations that are to be observed when discussing cross-reactivity, are at the resolution of the T cell receptor (TCR). TCRs are heterodimers comprised of subunits TCRα and TCRβ, though approximately 5% of human TCRs are comprised of TCRγ and TCRδ. Expression of TCRs and Ig chains on the surface of T cells is controlled by a mechanism known as allelic exclusion, with their expression corresponding to a single allelic copy [16]. This ensures that the *modus operandi* of T and B cell priming is that of clonal selection, the benefit of which entails avoidance of autoimmunity by way of high specificity [13]. However, biallelic expression of TRC and Ig kappa (κ) chain (Igκ) has been documented in T cells, and correlated with affinity for a broader spectrum of antigens [13], [159]. Of course, this alone cannot be attributed to cross-reactivity, as heterogenicity in T and B cell ligand receptor binding is now understood as putative [17]. Interestingly, incomplete allelic exclusion of the TCRα chain can lead to expression of two distinct TCRs, thereby increasing the likelihood of cross-reactivity [18].

Permissive and repressive epigenetic control of T cells, though an integral part of the canonical adaptive response, very likely extend towards the facilitation of heterologous immunity in the context of TCRs and surface Ig [147], [151], [160]–[166]. Though trained immunity is independent from T and B cells, heterologous immunity relies on the canonical relationship between the two components of the immune system. Perhaps characterised with heterogeny, BCG-induced cytokine

expression profiles predominantly include IFN-γ, which in turn stimulates macrophages and monocytes to secrete numerous cytokines, including IL-15. This cytokine regulates survival of T cells in the absence of antigens, either through induction of apoptosis or division [167]. It is possible that epigenetic alterations that occur upon BCG vaccination may influence permissiveness of the chromatin regions that corresponds to regulatory regions of the IL-15 gene, though this remains to be determined. In any case, the influence of BCG on heterologous immunity is likely predominantly mediated via innate immune cells. The threshold for TCR activation is lowered in effector/memory $CD8^+$ cells through the expression of TLR 1/2/6 and 6 are respectively. Considering that TLRs are important receptors in BCG recognition, this opens the possibility of epigenetic reprogramming as a result of TLR signalling.

Mycobacterial Activation of Bystander $CD8^+$ Cells

Activation of bystander $CD8^+$ cells, interestingly enough, is independent of TCRs, yet heavily dependent on secretion of IL-15, which BCG vaccination may indirectly induce [4]. Bystander activation of $CD8^+$ have been documented as the main sources of IFN-γ along with stimulated NK cells in melioidosis caused by *Burkholderia pseudomallei* [2]. Furthermore, enhanced expression of IFN-γ mRNA was documented in mouse models, however the study that reports this used and experimental *M. avium* model [3]. In spite of this, homologies between BCG and *M. avium* antigens may evoke similar, if not identical, T cell responses. Interestingly, virally activated $CD8^+$ exhibit strong affinity towards granulomas induced by BCG, though this has thus far only been documented for immunodeficient mice, and it is unclear whether BCG activation of T cells would have the same effect on viral infections [1]. In the absence of more comprehensive studies to draw a conclusion from, it may only be cautiously proposed that BCG-induced IL-15 secretion likely influences bystander $CD8^+$ T cell activation.

Though currently available evidence is somewhat suggestive of a relationship between non-specific T cell activation and BCG immunization, comprehensive work lies ahead in determining whether the vaccine may induce a $CD8^+$ cell phenotype that could contribute to better outcome with SARS-CoV-2. Considering that BCG contains a large number of highly diverse antigens, it is not surprising that T cells induced by BCG vaccination are quite broad in epitope specificity [110], [115], [168]. *Per contra*, excessive T cell cross-reactivity may lead to autoimmunity, thereby making the heterologous immunity narrative a double-edged sword [13]–[16], [18], [19]. Recently $CD4^+$ T cells cross-reactive to SARS-CoV-2 have been detected in COVID-19 patients, though the exact implications of this remain unclear, and are likely population-specific [169]. However, it has been proposed that their presence could potentially reduce viral loads in both the lungs and the upper respiratory tract upon infection.

Non-specific Immunomodulatory Effects of the BCG Vaccine

Reports of non-specific benefits of BCG vaccination on other infectious diseases has seen a steady increase in recent years, correlating the vaccine with reduced mortality rate among infants, along with adjuvant-like effects on other unrelated childhood vaccines [170]. Beneficial effects of BCG on non-mycobacterial infections is colloquially thought to be mediated by innate immune memory or heterologous lymphocyte activation [30], [171], due the absence pathogen-specific antibody epitopes in mouse studies where the vaccine conferred a better outcome in infections with *Salmonella typhimurium* and challenges with *Plasmodium spp.* and *Babesia* [172]. Perhaps the most striking evidence regarding non-specific BCG benefit is the improved antibody response to oral polio vaccine boosting detected in patients who were also given BCG at the time of booster administration [173], [174]. Thus, it is likely that the beneficial effects of the BCG vaccine vary concordantly to the strain of BCG and the immunogenetic background of the host. For instance, an Australian study conducted on 56 BCG-vaccinated and 52 BCG nonvaccinated infants, uncovered higher titters of IgG with epitopes for *Haemophilus influenzae* type B polysaccharides, pneumococcal capsular polysaccharide PAMPs and tetanus toxoid (TT) [175]. *Per contra*, a randomized study on new-borns in Denmark found that a reduction in infant hospitalizations was only for cases where the mothers were also BCG vaccinated [176], [177].

Non-specific BCG effects do not shy away from the domain of respiratory viral infections,

where beneficial effects of BCG continue to be reported, however comprehensive understanding of this puzzling occurrence is modest at best [8], [31], [120]. Although a number of different BCG strains exist and continue to be regularly used, there is very limited work on the efficacy conferred by each different strain, both in tuberculosis prophylaxis and non-specific effects in non-

Table 1. Some currently ongoing clinical trials regarding the correlation of the BCG vaccine and reduced risk of COVID-19 (https://clinicaltrials.gov/)

Title	Status	Interventions	Locations
Reducing Health Care Workers Absenteeism in Covid-19 Pandemic Through BCG Vaccine	Active, not recruiting	– Drug: BCG Vaccine – Drug: Placebo	– Jeroen Bosch ziekenhuis, Den Bosch, Brabant, Netherlands – Canisius Wilhelmina Ziekenhuis, Nijmegen, Gelderland, Netherlands – Radboud UMC, Nijmegen, Gelderland, Netherlands – Sint Maartenskliniek, Nijmegen, Gelderland, Netherlands – Noordwest Ziekenhuisgroep locatie Alkmaar, Alkmaar, Noord Holland, Netherlands – Hagaziekenhuis, Den Haag, Zuid-Holland, Netherlands – Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands – Erasmus Medical Center, Rotterdam, Zuid-Holland, Netherlands – University Medical Center Utrecht, Utrecht, Netherlands
Reducing COVID-19 Related Hospital Admission in Elderly by BCG Vaccination	Active, not recruiting	– Biological: BCG vaccine – Biological: Placebo	– Radboud University, Nijmegen, Gelderland, Netherlands – UMC Utrecht, Utrecht, Netherlands
BCG Vaccination for Healthcare Workers in COVID-19 Pandemic	Active, not recruiting	– Biological: Bacille Calmette-Guérin (BCG) – Other: Placebo Comparator	– TASK Foundation, Cape Town, Western Cape, South Africa
BCG Vaccination to Protect Healthcare Workers Against COVID-19	Active, not recruiting	– Drug: BCG Vaccine – Drug: 0.9%NaCl	– St Vincent's Hospital, Sydney, Sydney, New South Wales, Australia – Prince of Wales Hospital, Sydney, New South Wales, Australia – Sydney Children's Hospital, Randwick, Sydney, New South Wales, Australia – The Children's Hospital at Westmead, Sydney, New South Wales, Australia – Westmead Hospital, Sydney, New South Wales, Australia – Royal Adelaide Hospital, Adelaide, South Australia, Australia – Women's and Children's Hospital, North Adelaide, South Australia, Australia – Royal Children's Hospital, Melbourne, Victoria, Australia – Epworth Richmond, Melbourne, Victoria, Australia – Monash Health- Monash Medical Centre, Melbourne, Victoria, Australia – and 26 more
Prevention, Efficacy and Safety of BCG Vaccine in COVID-19 Among Healthcare Workers	Active, not recruiting	– Biological: BCG vaccine – Other: Placebo	– Hospital Universitario "José E. González", Monterrey, Nuevo León, Mexico
BCG Vaccine in Reducing Morbidity and Mortality in Elderly Individuals in COVID-19 Hotspots	Active, not recruiting	– Biological: BCG vaccine (Freeze-dried)	– Tuberculosis Research Centre, Chennai, Tamilnadu, India
Efficacy and Safety of VPM1002 in Reducing SARS-CoV-2 (COVID-19) Infection Rate and Severity	Active, not recruiting	– Biological: VPM1002 – Other: Placebo	– University Health Network, Toronto, Ontario, Canada

mycobacterial infectious diseases. There is some evidence indicating that BCG decreases the morbidity of acute lower respiratory tract infections caused by respiratory syncytial virus (RSV); this effect was observed in young children in Guinea-Bissau and in a study that included elderly people, where a decrease in incidence of acute upper respiratory tract infections was reported [178]. However, it should be noted that the study on elderly people, which did yield positive results in favour of the non-specific protection of BCG against viral infections, was conducted by administering the vaccine once a month for three months, thereby warranting cautious interpretation. A significant reduction in respiratory tract infections was also reported in a study of BCG-vaccinated adolescents in the South-African population [179]–[181]. Although the results of these studies are in favour of non-specific prophylactic BCG effects in viral infections, therapeutic effects of the vaccine have also been reported, specifically regarding patients infected with the human papilloma virus (HPV) [182].

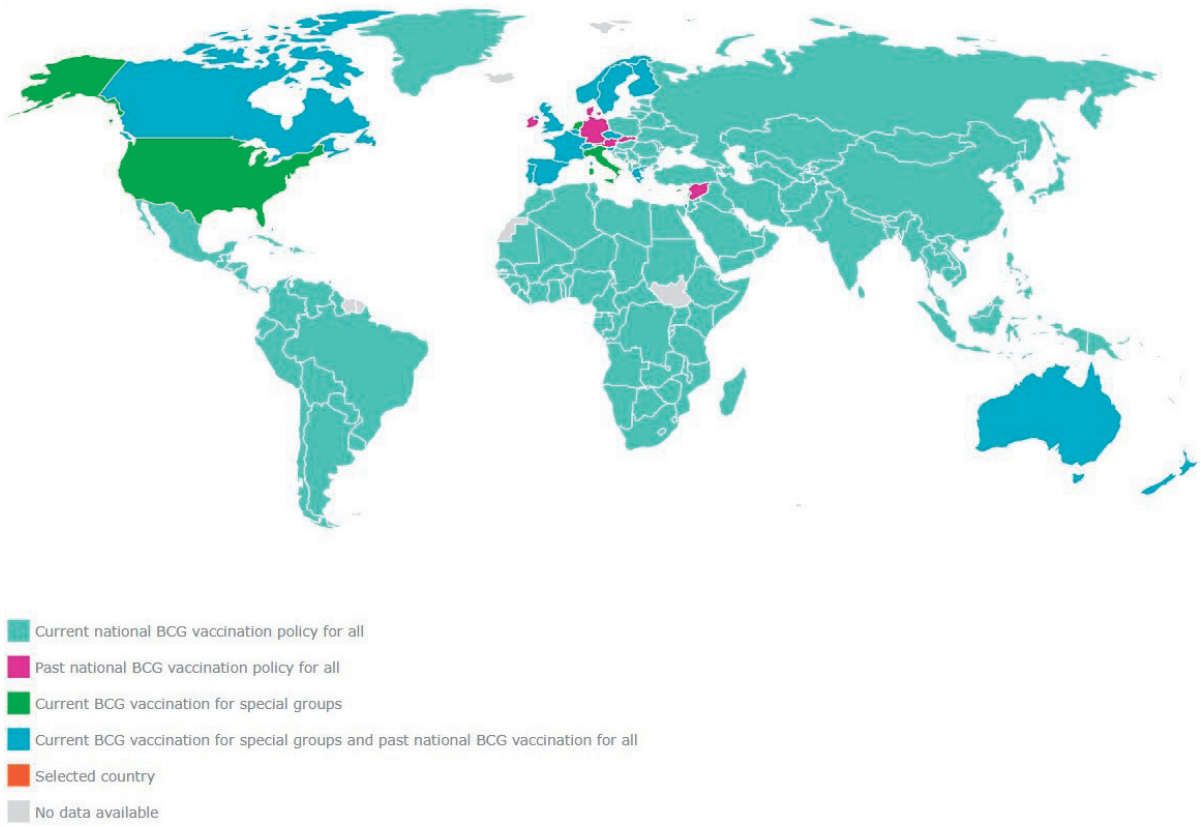
Perhaps most interesting for the COVID-19 pandemic is a study that reported improved antibody titters for the influenza A strain (H1N1) that caused the 2009 "swine flu" epidemic, when BCG was administered prior to the H1N1 vaccine. The enhanced protection was hallmarked by an improved production of IFN-γ for the H1N1 study [183], contrasted with another study that reported that BCG-induced IL-1β production is the likely mechanism of conferred protection during viral infections [151]. However, the same IFN-γ-mediated protection was observed for the vaccinia virus in infected mice upon BCG vaccination, which promoted the secretion of this cytokine by $CD4^+$ T cells [184]–[186]. IL-1β plays a role in inflammatory responses, apoptosis, cell differentiation and proliferation, and has shown to play an important role in viral immunity [187]. Improvement of non-specific Th1 and Th17 immune responses, along with enhanced innate trained immunity, has also been reported in BCG-immunized patients, with satisfactory durations [188].

Implications for the COVID-19 Pandemic

At the present moment, there are a number of clinical trials aimed at evaluating the presumed protective effects of the BCG vaccine towards COVID-19, some of which are summarized in **Table 1**. It is likely, however, that BCG strain will have an impact on its effects on COVID-19 and infections with SARS-CoV-2, and trials are currently underway for the purpose of assessing which strain, if any, is adequate for implementation in the battle against COVID-19. Thus far, the candidates of interest are the Danish and Tokyo strain, although it currently remains utterly unclear what the immunological basis for their difference in efficacy might be [106]. Frequently used BCG strained along with their characteristics are summarized in **Table 2** [189], [190]. Virulence of the BCG strain was hypothesized to play a role in protection against *Mycobacterium tuberculosis*, potentiating the assumption that the trials will eventuate in varying efficacy across BCG strains for COVID-19 [43]. There is an obvi-

Table 2. Summarization of frequently used BCG vaccines and their characteristics. Abbreviations: CRR, complete response rate; NA, not applicable

Strain	Mean CRR	Weight (mg)	Recommended dose (cfu)[±]	Secretion of lipid virulence factors	Secretion of MPB64/ MPB70 and MPB8
RIVM/1	60	80	2-30 x 10^8	NT	Unknown
Romanian	64	NA	NA	NT	Unknown
Copenhagen	67	NA	NA	Yes	Absent/Present
S. African	69	NA	NA	NT	Unknown
A. Frappier	60 (39–100)	NA	NA	Yes	Absent/Present
Glaxo	65 (53–88)	NA	NA	No	Absent/Present
Tice	71 (56–82)	12.5	2-8 x 10^8	Yes	Absent/Present
Pasteur	74 (40–80)	NA	NA	Yes	Absent/Present
Tokyo	77 (63–84)	80	0.4-0.5 x 10^8	No	Present/High
Connaught	79 (70–92)	81	1.8-15.9 x 10^8	NT	Unknown
Moreau RdJ	90	80	0.04 x 10^8	No	Present/High
Moscow	90.5	120	3-57 x 10^8	Yes	Present/High



ous need to bridge the gap between findings in basic immunobiology and clinical application, which will hopefully occur upon the conclusion of these clinical trials. Since COVID-19 vaccines are still not internationally widely available to all countries, the potential for utilization of existing vaccine technologies in mitigating some of the fallout caused by COVID-19, could significantly improve patient care in countries where COVID-19 vaccines are scarcely available.

Concluding Remarks

Although the mechanisms of action remain unclear, non-specific effects of BCG have been reported to confer a degree of protection against viral infections and non-mycobacterial bacterial infections. It is likely that BCG predominantly influences innate immune memory, causing epigenetic modulation of monocytes and macrophages, and inducing secretion immunomodulating cytokines. Effects on heterologous immunity, however, can currently only be described as indirect, particularly through induction of IL-15 secretion. Though heterologous immunity does, indeed, depend on adequate pathogen processing by the innate immune system, BCG-induced bystander $CD8^+$ activation may boost the innate immune response through enhanced IFN-γ production.

Although recent studies have shown that BCG promotes the Th1 and Th17 immune response, further studies should be directed at uncovering whether such effects are dependent on BCG strain and the immunogenetic background of patients. Considering that BCG has been shown to increase titters of polysaccharide-specific IgG in bacterial infections, it is possible that the same might be true in the case of SARS-CoV-2 spike glycoprotein, which the virus uses to bind to cells expressing ACE2 and C-type lectins. Substantial evidence has mounted over the years in favour of non-specific benefits of BCG vaccination in a variety of other infectious and oncologic diseases, further solidifying the plausibility of this model. However, the effects of this vaccine vary in accordance to BCG strain and likely a plethora of host-derived factors, most of which are incompletely understood. In essence, trained and heterologous immunity are incredibly complex and multifaceted phenomena with proven therapeutic potential, and could possibly confer improved outcome in asymptomatic SARS-CoV-2 infec-

tions, or COVID19 disease. It is encouraging to see the number of clinical trials that are currently underway, tasked with resolving a lot of unclarity regarding the issue of BCG-induced heterologous immunity. Though this work is concerned with providing a hypothetical model by which the BCG vaccine may induce non-specific protection against SARS-Cov-2 – based on studies concerning other pathogens – we recognize the limitations of all previous studies pertaining to this topic. Firstly, though this is a plausible immunobiological model, it largely based on *in vitro* and animal studies, with several prominent examples derived from human test subjects. Human studies concerning heterologous immunity tend to suffer from the issue of bias, and the potentially relevant host-derived intricacies influencing the ability of a vaccine to influence innate immune cell memory, is difficult to control for. Though our model holds plausibility, it should be understood as suggestive rather than definitive, and more work is definitely needed – one that bridges clinical relevance and basic immunobiological studies – in order to derive a definitive conclusion.

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REVIEW PAPER

JMS Journal of Medical Science

Cardiovascular risk factors of poor prognosis in COVID-19 – a review

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ABSTRACT

Since the first report in 2019, COVID-19 has claimed many lives, even those previously in good health. Therefore, a proper diagnosis and identification of patients at the highest risk of serious complications is vital. In fact, COVID-19 can lead to systemic inflammation and multiorgan dysfunction. Apart from the respiratory system, the circulatory system is also affected, including numerous complications due to the cytokine storm, direct cytotoxic effects, downregulation of angiotensin-converting enzyme 2, and low oxygen blood levels. In this review, we discussed cardiovascular risk factors associated with a poor prognosis in COVID-19 patients, including pre-existing risk factors or those acquired in the course of the infection. We also analyzed the role of biomarkers, ECG, and imaging in the identification of patients at the highest risk of unfavorable outcomes, as even subtle abnormalities in additional tests may have a significant impact on disease management.

Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It was first reported in Wuhan, China, in December 2019 and quickly escalated into a global pandemic declared by the World Health Organization on March 11th, 2020.

SARS-CoV-2 and the cardiovascular system

SARS-CoV-2 is an enveloped, positive-sense single-stranded RNA virus (+ssRNA) from the *Betacoronavirus* genus which includes severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV), i.e. diseases with their own pan-

demics. In fact, SARS-CoV-2 was shown to share many similarities with SARS-CoV, including their mode of entry and nearly 80% of the genomic sequence.

In order to gain entry into human cells, the virus uses a spike protein to bind to a cellular receptor angiotensin-converting enzyme 2 (ACE2). This receptor is found in various organs, such as the lungs, heart, kidneys, and intestines [1, 2], and this fact could account for the association of SARS-CoV-2 with cardiovascular (CV) complications [3].

ACE2 degrades the product of ACE, angiotensin II, and converts it into angiotensin 1-7, which has anti-hypertrophic, anti-fibrotic, vasodilatory, and anti-hypoxic effects on the heart. Thus, ACE2 acts as a counterbalance to the renin-angiotensin-aldosterone system, and participates in cardioprotection [4].

In addition to the respiratory and CV manifestations, SARS-CoV-2 can trigger thromboembolic events and a cytokine storm [3]. Cytokine storm refers to the uncontrolled immune cell activation and overproduction of pro-inflammatory cytokines. Furthermore, it increases the levels of reactive oxygen species and causes endothelial cell dysfunction, disruption of blood supply, and multiple organ failure [5]. This, in turn, could further enhance the spreading of SARS-CoV-2, as ACE2 was found to be an interferon-stimulated gene [1].

Therefore, the CV system could be affected in a number of ways, potentially in synergy through (a) direct myocardial injury resulting from viral binding to ACE2 and the subsequent downregulation of ACE2 expression, (b) systemic inflammation resulting in the organ failure, increase in the myocardial demand-supply ratio, atherosclerotic plaque rupture, as well as electrolyte imbalance, (c) decreased blood oxygen levels as a result of pulmonary damage, and (d) COVID-19 therapies which negatively affect the CV system – including antiretroviral therapy, azithromycin and tocilizumab [3, 5]. The impact of SARS-CoV-2 on the CV system is presented in **Figure 1**.

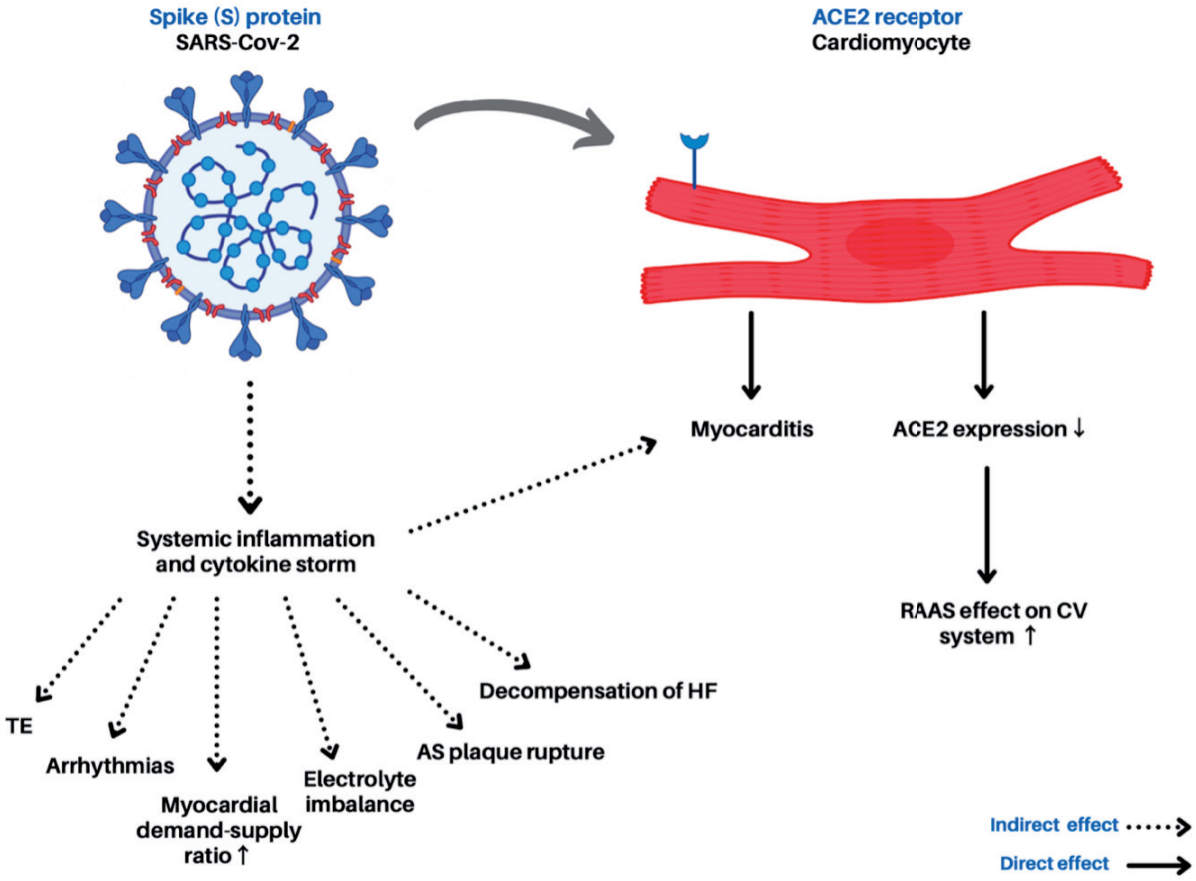

Figure 1. The impact of SARS-CoV-2 on the cardiovascular system. ACE2 – angiotensin-converting enzyme 2, AS – atherosclerosis, HF – heart failure, RAAS – renin-angiotensin-aldosterone-system, TE – thromboembolism

Due to the tropism of SARS-CoV-2 towards the heart and its ability to exacerbate, or even cause CV disease, it is vital to monitor the hearts of patients suffering from COVID-19, particularly those with CV comorbidities, since they are more likely to develop severe presentations of COVID-19. Moreover, diabetes, hypertension, and cerebrovascular diseases were also associated with a higher risk of severe COVID-19 presentations [2]. The aforementioned non-communicable diseases, in addition to cancer and chronic respiratory disease, constitute a syndemic with COVID-19 posing a significant strain on our healthcare systems and increasing the risk of premature death [6].

The link between the treatment with angiotensin-converting enzyme inhibitors, or angiotensin II receptor blockers, and the upregulation of ACE2 expression is controversial, with studies showing inconsistent and mixed results [7–11]. Angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers do not have a direct effect on ACE2, and there is no evidence of COVID-19 susceptibility stemming from ACE2 upregulation. Therefore, several medical societies have recommended the continuation of treatment with those drugs [2].

Factors of poor prognosis in COVID-19

Cardiovascular complications

CV complications of COVID-19 include myocardial injury, myocarditis, acute myocardial infarction (AMI), heart failure (HF), arrhythmias, and venous thromboembolic events [12].

COVID-19 may disproportionately affect individuals suffering from CV diseases, in particular patients with coronary artery disease (CAD) and HF. A meta-analysis which analyzed 22,148 patients from 40 studies showed that the underlying CV disease in COVID-19 patients was correlated with a poorer prognosis, including a more severe course of COVID-19, intensive care unit (ICU) admissions, and disease progression [13].

Myocarditis

Myocarditis constitutes a potentially serious cardiac complication of COVID-19. It refers to the inflammation of the myocardium characterized by inflammatory infiltrates and myocardial injury without an ischemic cause. Direct viral injury and further cardiac damage due to the body's immune response to the virus are believed to be the pathophysiologic causes underlying COVID-19-related myocarditis. Although it is not an uncommon complication, the true incidence of myocarditis in COVID-19 patients remains unknown. This is due to its highly variable clinical presentation, lack of reliable laboratory tests, and non-sensitive or specific findings on ECG. In fact, patients usually require further testing in order to establish a diagnosis of myocarditis using either echocardiography, cardiac magnetic resonance imaging (CMR), or endomyocardial biopsy [14, 15].

Symptoms of COVID-19-related myocarditis are most commonly mild [15]. However, if myocardial dysfunction develops, the prognosis becomes poor [16]. Patients with comorbid cardiac diseases and those with concomitant elevated troponins tend to present worse outcomes [15]. The management is focused on supportive care with fluids, remdesivir, and close monitoring. Additionally, patients who develop cardiogenic shock (CS) will require inotropes, mechanical ventilation, and possibly temporary mechanical circulatory support [14, 15].

Ischemic heart disease

Patients without prior cardiovascular disease

Several articles reported evidence of AMI type 2, or myocardial infarction with non-obstructive coronary arteries among patients with COVID-19. The former is explained by the presence of high levels of ACE2 receptors in pericytes and in endothelial cells, which inhibits a severe microvascular dysfunction also associated with cytokine storm [17].

Acute myocardial infarction

Although in certain areas a decrease of up to 50% in AMI cases was reported during the COVID-19 pandemic [18, 19], AMI has been recognized as a severe cardiac complication in COVID-19 patients, and can also develop in individuals without prior CV conditions. This is possibly accounted for by the extensive inflammation and hypercoagulability resulting from the disease. Furthermore, a direct viral injury of myocardial cells and oxygen imbalance caused by pneumonia are additional potential contributors [20, 21].

One study suggested investigating inflammatory markers and N-terminal pro-B type natriuretic peptide (NT-proBNP) in COVID-19 patients with concomitant AMI, as these were shown to be elevated and can be effective in determining the disease severity. The same study demonstrated higher mortality from COVID-19 in patients experiencing AMI compared to those without AMI [22]. Even after undergoing percutaneous coronary intervention for STEMI – in-hospital mortality, stent thrombosis, and CS remain significantly higher in COVID-19 patients [23].

Myocardial infarction with nonobstructive coronary arteries

Myocardial infarction with nonobstructive coronary arteries is clinically defined in patients who fulfil the universal AMI criteria, yet have less than 50% stenosis in coronary angiography [24]. One study investigated patients with both obstructive and nonobstructive coronary arteries who suffered AMI in the course of an underlying COVID-19 infection. Both their in-hospital mortalities were high, and no difference was found between the two groups of patients [25].

Patients with prior coronary artery disease

According to the findings of Zhou et al., CAD is a risk factor of increased mortality in the course of COVID-19 [26]. However, a study conducted in Italy on 1,252 COVID-19 patients demonstrated that age and female gender were the only independent correlates of mortality (where age was a risk factor and female gender was a protective factor). Patients with CAD presented a poorer prognosis, although this was mainly attributed to older age and a higher rate of comorbidities rather than to a direct consequence of CAD [27]. In another study from Italy, COVID-19 patients who underwent high-resolution computed tomography (CT) were retrospectively evaluated by the coronary calcium score (CCS). Only patients with subclinical CAD were included in the study, and out of 53 patients analyzed, 50% of individuals with CCS ≥ 400 had died, compared to only 8.9% of patients with a CCS < 400. The independent predictive role of CCS could not be ultimately determined due to a very small study group. However, a high CCS score can be a marker of worse in-hospital outcomes, although it may also indicate an increased baseline risk. Nevertheless, including a CCS assessment in a routine high-resolution CT evaluation in COVID-19 patients can deliver useful prognostic information without additional costs [28].

Heart failure

Patients with congestive HF have a significantly poorer prognosis in the course of COVID-19. In fact, certain cases have been observed where COVID-19 has caused a decompensation of underlying HF, leading to CS [29]. A retrospective case series conducted in Wuhan, China, further consolidates the importance of early cardiac monitoring in patients with congestive HF, due to the high correlation between the disease and mortality [30]. However, acute HF also occurred in patients without underlying HF, leading to a significantly higher mortality (46.8% vs. 19.7%; $p < 0.001$) [31].

Arrhythmias

Arrhythmias are not uncommon in viral infections and were also demonstrated in COVID-19 patients. The most probable causes comprise such elements as fever, stress, hypoxia, electrolyte imbalances, and usage of antiviral drugs. In Wuhan, China, over 44% of ICU COVID-19 patients presented arrhythmias. Furthermore, sinus bradycardia is one of the most common arrhythmias seen in COVID-19 patients and it can persist for up to 2 weeks [32]. A case study following two patients with COVID-19 related bradycardia demonstrated a positive response to epinephrine, deeming temporary pacing unnecessary [33]. Interestingly, atrial fibrillation is also widely reported. The treatment is focused on rhythm control and anticoagulation simultaneously avoiding non-pharmacological interventions. Thus, catheter ablation and electrical cardioversion should be avoided, if possible, during an active infection [34]. Other atrial and ventricular arrhythmias, including ventricular fibrillation, were also reported in patients who showed no previous evidence of arrhythmia and were not on QT-prolonging medications. It is also essential to monitor COVID-19 patients with continuous telemetry, as the development of new-onset arrhythmia is associated with a severe course of the disease [32].

In addition, some patients suffering from inherited arrhythmias, such as long-QT syn-

drome, Brugada syndrome, short-QT syndrome, and catecholaminergic polymorphic ventricular tachycardia, may be at a higher risk of developing arrhythmias following SARS-CoV-2 infection [35].

Chloroquine, a medication primarily used to prevent and treat malaria, was administered to COVID-19 patients early in the pandemic, as it interferes with the terminal glycosylation of ACE2, which may disturb virus-receptor binding and thus prevent infection. The use of chloroquine and its derivative hydroxychloroquine was halted in summer 2020 due to the lack of a benefit seen in the randomized clinical trials and the potential for toxicity. Although chloroquine and hydroxychloroquine have QT-prolonging effects, they are mostly modest and do not require special attention in the long-QT syndrome. However, since they are metabolized by CYP3A4, these substances should be used with caution when in combination with other antiviral medications inhibiting CYP3A4, such as ritonavir-lopinavir, azithromycin, or remdesivir [35].

It is generally accepted that sodium channel function is sensitive to temperature. Fever stemming from COVID-19 can thus disturb the mutant sodium channels in Brugada syndrome and trigger arrhythmias. In fact, the presence of a pathogenic variant in SCN5A was shown to be of particular importance for Brugada syndrome patients who develop life-threatening arrhythmic events in the setting of fever. Hence, it is vital for Brugada syndrome patients to receive antipyretics immediately if they develop a fever [35].

In terms of the short-QT syndrome, it is extremely rare without any specific triggers for life-threatening arrhythmic events. Therefore, patients experiencing it are not expected to be at risk when infected with SARS-CoV-2 [35].

It is worth bearing in mind that exercise and stress are specific triggers for life-threatening arrhythmic events in catecholaminergic polymorphic ventricular tachycardia. Fever, however, is not. Hence, the risk of COVID-19 arrhythmias in those patients is controversial. It is important to avoid drugs with alpha- or beta-adrenoceptor mimetic activity, such as epinephrine during hemodynamic support, due to their ability to unmask ventricular arrhythmias in catecholaminergic polymorphic ventricular tachycardia patients [35].

Thrombotic complications

The mechanisms of increased thrombotic complications in COVID-19 patients are not exactly understood. However, any of the three factors in Virchow's triad may contribute to thrombosis. COVID-19 can result in hypoxia, leading to increased blood viscosity, and abnormal blood flow. Additionally, SARS-CoV-2 also causes a state of increased inflammation, resulting in a hypercoagulable state due to a release of inflammatory cytokines [30]. As the virus enters cells through the ACE2 receptor, also found in the endothelial cells, it triggers the production of mitochondrial reactive oxygen species, glycolytic shift, and endothelial damage. In fact, spike protein alone can damage endothelium by down-regulating ACE2 and as a consequence inhibiting mitochondrial function [36].

Among different negative prognostic factors, a high D-dimer score on admission has been reported to be a significant risk factor for severe disease course and mortality [36].

A retrospective cohort study from China demonstrated that D-dimer levels above 1 μg/mL increased the chance of in-hospital death [26]. Another study from Wuhan further consolidated this statement indicating that a high D-dimer, high fibrin degradation products, longer prothrombin time, and activated partial thromboplastin time during admission were common in patients who died [37].

In addition, another study revealed that, although not helpful to all patients, heparin administration decreased the 28-day mortality in patients with a sepsis-induced coagulopathy score ≥ 4, or D-dimer > 6-fold of the upper limit of normal [38].

D-dimer levels can be used to exclude a pulmonary embolism (PE) in case of a normal value, but it is not recommended as a positive marker, due to low specificity. Bompard et al. suggest using contrast-enhanced CT to exclude PE, if supplementary oxygen is required in infected patients [39].

PE, as well as thrombotic complications in general, constitute greater threats and are tremendously more common in the ICU patients than in the non-ICU patients. A study conducted in the Netherlands enrolled 184 patients with proven COVID-19 pneumonia that were admitted to the ICU. 23 of them died, 22 were discharged home,

and 139 were still in the ICU by the end of the study. Despite receiving at least standard-dose thromboprophylaxis, 31% (95% CI 20-41) of patients suffered thrombotic complications, with PE being the most common one (n = 25.81%). The independent predictors of thrombotic complications were spontaneous prolongation of the prothrombin time (> 3 s) and activated partial thromboplastin time (> 5 s). These findings emphasize the importance of high-dose prophylactic anticoagulation in all ICU patients suffering from COVID-19 [40].

Biomarkers

Multiple studies hypothesized that measurement of cardiac damage biomarkers immediately after hospital admission for SARS-CoV-2 infection, followed by longitudinal monitoring during hospitalization, could help clinicians identify a subset of patients with a possible cardiac injury and predict the progression of COVID-19 towards a poorer outcome [41].

The prognostic value of cardiac biomarkers has been widely used in the management of CAD and HF. Troponins, B-type natriuretic peptide (BNP), and NT-proBNP are most commonly employed for the diagnosis, prevention, and safe discharge planning. Troponin is a marker of direct myocyte damage and necrosis, whereas BNP and NT-pro BNP are rather defined as markers for myocardial stretch injury. They can be also used in terms of the diagnosis, prevention, and safe discharge planning for patients hospitalized with CV diseases [42].

Troponin I (TnI) constitutes a "gold standard" biomarker of necrosis used for the cardiac risk assessment. It is released exclusively in the cardiac muscle in the presence of myocardial injury irrespective of its mechanism [43]. Interestingly, even in the absence of acute coronary syndrome (ACS), troponin elevation was identified in 20–30% of hospitalized patients with COVID-19 and has been associated with an increased risk of mortality in retrospective studies [44, 45]. A study from Zhou et al. on 191 patients, of whom 54 deceased due to COVID-19, revealed increased troponin I in over half of those who died. 91 (48%) of the patients had comorbidities, such as hypertension (most common), diabetes mellitus, and CAD [26]. A cohort study by Shi et al., including 416 hospitalized COVID-19 patients, reported that approximately 20% of the subjects had evidence of a cardiac injury manifested by a significantly elevated high sensitivity troponin I (hs-TnI). This finding, in turn, was associated with a higher in-hospital mortality (51.2% vs. 4.5% respectively; p < 0.001) [43, 44]. Similarly, a cohort study by Salvatici et al. on 523 patients with COVID-19 reported a 18.3% mortality during hospitalization, and significantly higher hs-TnI levels in the deceased patients in comparison to the survivors (36.05 ng/L IQR 16.5–94.9 vs. 6.3 ng/L IQR 2.6–13.9; $p < 0.001$ respectively) [46].

Furthermore, Singh et al. conducted a single-centered, retrospective, observational study on 276 patients who presented to the emergency department. In 261 (95%) patients high sensitivity troponin T (hs-TnT) values were noted at presentation. The median initial hs-TnT value was 17 ng/L. In fact, initial hs-TnT levels above median were associated with longer hospitalization, increased need for vasoactive agents, higher mortality, along with the composite end-point (in-hospital death, cardiac arrest, intubation, or need for critical care); (OR 3.92, p < 0.001). From this patient group, only one (< 1%) with elevated hs-TnT had clinical evidence of ACS and underwent percutaneous coronary intervention. This finding supports the observation of Tersalvi et al. according to which elevated troponin levels are most likely the manifestation of an inflammatory response rather than true MI [41].

In addition to troponins, creatine kinase-MB (CK-MB) may also hold prognostic value in COVID-19 patients. It is an intracellular enzyme present in the skeletal muscle, myocardium, and brain. In the study conducted by Wang et al., 36 out of 138 patients (26.1%) were admitted to the ICU. All the patients had significantly elevated TnI and CK-MB levels compared to the non-ICU patients, which indicates that myocardial injury is more severe in cases with a serious course of COVID-19 [47].

Myoglobin is a cytoplasmic protein which exists in the cardiac and skeletal muscle. It increases rapidly and is among the initial markers to be elevated. In the study by Yang et al., the levels of myoglobin in the critically ill COVID-19 patients were significantly higher than in the mildly affected patients. In a cross-sectional study by Yu et al. on 162 patients requiring ICU, myoglobin was elevated in 57 (35.2%) patients.

Even though myoglobin is not as cardiac-specific as troponins, it was positively correlated with CK-MB and troponin T [47, 48].

A multicenter observational study was conducted at Sichuan province and Wuhan city to establish the predictive value of biomarkers on 357 patients with confirmed COVID-19 infection from January to March, including 22 tertiary hospitals designated for COVID-19 patients in the area. After a 28-day follow up, patients were classified into survival (n = 332) or death groups (n = 25), and recovery (n = 314) or non-recovery (n = 43) groups. Myoglobin, CK-MB, and hs-TnT were significantly elevated in death and non-recovery groups. Least absolute shrinkage and selection operator regression (a machine learning regression which chooses the independent risk factors affecting outcomes and presents only the strongest predictors in the predictive model) was employed by Yang et al. in order to identify the strongest predictive biomarkers. The area under the curve (AUC) of myoglobin and CK-MB for in-hospital death were 0.838 (95%CI: 0.729–0.947, $p < 0.001$) and 0.862 (95%CI: 0.804–0.920, $p < 0.001$), respectively. The AUC of myoglobin and CK-MB for non-recovery were 0.841 (95%CI: 0.765–0.918, $p < 0.001$) and 0.839 (95%CI: 0.786–0.892, $p < 0.001$), respectively. Myoglobin and CK-MB were considered as possible adverse prognosis predictors regarding in-hospital death and non-recovery in 28 days. In contrast, the method did not demonstrate the predictive value of hs-TnT in this study, whereas a combined use of CK-MB and myoglobin showed better predictive performance in terms of the prognosis [47, 48].

NT-proBNP is secreted in response to increased myocardial wall stress. Previous studies suggested that NT-proBNP could be a powerful predictor of mortality in community-acquired pneumonia The elevated NT-proBNP levels are claimed to be the result of cardiac complications following complex interactions among pre-existing conditions, relative ischemia, up-regulation of the sympathetic system, systemic inflammation, and direct pathogen mediated damage to the CV system [49, 50]. A study by Gao et al. revealed that NT-proBNP correlated independently with in-hospital death of COVID-19 patients. The cut-off value of NT-proBNP to predict the fatal outcome of the disease was > 88.64 pg/ml, and was significantly decreased as compared to the threshold used to diagnose HF (450 pg/mL for < 50 years old, 900 pg/mL for 50–75 years old and 1800 pg/mL for >75 years old) [49].

Lactate dehydrogenase (LHD) is an intracellular enzyme found in most cells. Although LHD can be used as a marker for cardiac damage, abnormal values can also be seen with multiple organ injuries. Henry et al. showed that elevated lactate dehydrogenase values in 1532 COVID-19 patients were associated with a > 6-fold increase in odds of severe course of disease. Moreover, a > 16-fold increase in the odds of mortality was also observed. Elevated levels of LHD measured at the earliest time point during the hospitalization were found in > 95% deceased patients and < 60% of survivors. Therefore, since elevated LHD levels reflect multiple organ injuries, they may play a prominent role in the triage of patients with COVID-19 [51, 52].

It is also of importance to note that in terms of the massive proinflammatory and prothrombotic cytokine storm associated with COVID-19 infection, not only cardiac biomarkers are increased, but also many others, e.g. IL-6, CRP, ferritin and D-dimers [26, 53, 54].

ECG

ECG is a widely available tool which monitors the electrical activity of the heart and is often used to aid the diagnosis and stratify the risk in heart diseases. A study which continuously monitored the ECGs of 159 COVID-19 patients on admission for 7 days, found a significant correlation between abnormal ECG and major adverse events. 49.1% of patients had abnormal ECG findings on admission, and 53.5% at day 7. Ischemic changes and left ventricular hypertrophy correlated with a higher risk of major adverse events. The multivariable analysis demonstrated that abnormal ECG on the 7th day of hospitalization was an independent predictor of major adverse events (HR 3.2, 95% CI 1.2-8.7; p = 0.02). In addition, patients with irregular ECGs at day 7 were more likely to need renal replacement therapy and an ICU admission. The study also found that a high heart rate and its increase at day 7 could point to a strong systemic inflammatory reaction, whereas low QRS voltages could indicate significant lung damage, and the widening of QRS complex at the time of hospital stay could be significant of direct myocardial injury [55].

Another study followed the ECGs of 50 patients with COVID-19 pneumonia. On admission, 63% experienced either left ventricular hypertrophy or ST-T abnormalities. Another 26% developed new ECG irregularities, with changes indicating acute pericarditis being the most prevalent (12%). This may be due to the expression of ACE2 receptors in epicardial adipocytes, which were associated with atrial electrical remodeling and progression to atrial fibrillation. This suggests that involving the epicardial adipocytes in COVID-19 patients (for instance, by developing pericardial effusion) may increase the risk of developing atrial fibrillation [56]. Possible ECG abnormalities in the course of COVID-19 infection are presented in **Figure 2**.

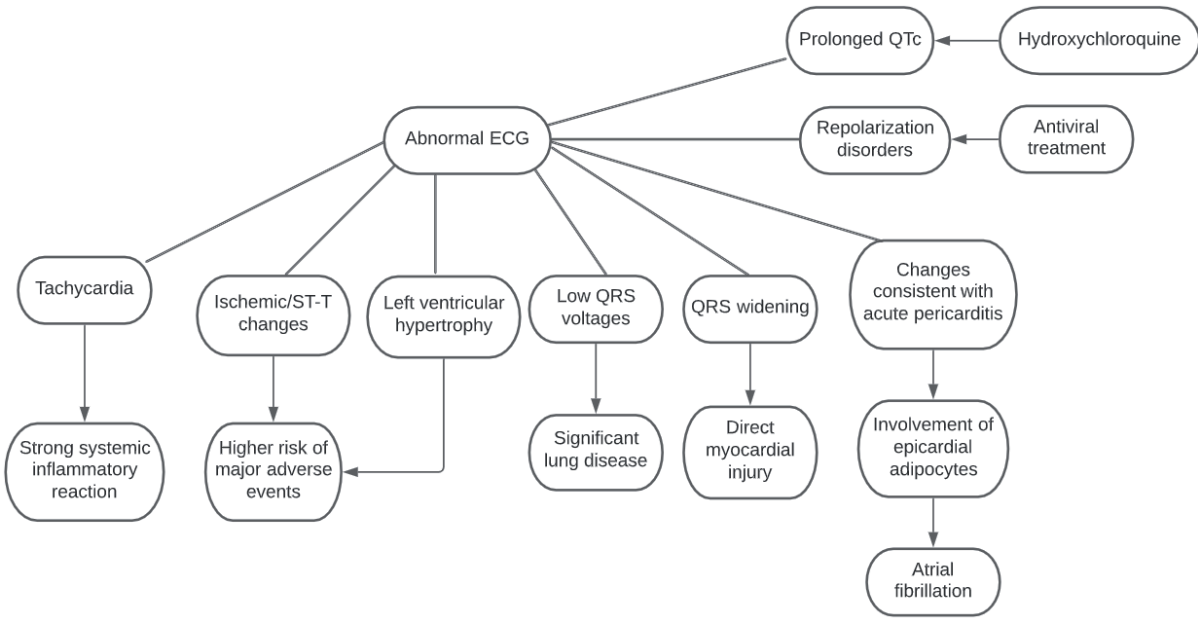

Figure 2. ECG changes in COVID-19 [on the basis of 55, 56]

Cardiovascular imaging

As cardiac involvement in COVID-19 is common and leads to an increased mortality, imaging techniques play a pivotal role in the differential diagnosis and risk stratification of CV manifestations [5]. Bedside ultrasound assessment of the heart and vessels is an effective first-line tool in detecting any abnormalities, whereas advanced techniques may facilitate final diagnosis and decision-making. In fact, echocardiography is frequently used in the initial assessment of acute myocardial injury. However, both logistic and sanitizing problems limit the use of CMR and CT. Even in echocardiography, simplified protocols are preferred to reduce exposure, while advanced analysis can be performed in post-processing [57].

Echocardiographic examination in patients with COVID-19 allows for a hemodynamic evaluation, as well as for the identification of typical features of myocarditis, HF, ACS, or PE [58]. According to Cresti et al., left ventricular dysfunction, fortunately often reversible, is common even among patients without prior CV disease [57]. In a prospective international survey by Dweck et al., 55% of patients with suspected or confirmed COVID-19 (n = 1.216) demonstrated an abnormal echocardiogram (including 46% without pre-existing heart disease; n = 901) [59]. In another study, 26% of COVID-19 patients (n = 125) showed left ventricular impairment, defined as a left ventricular ejection fraction < 50%, or segmental wall motion abnormalities [60]. However, it is postulated that it is the right ventricular (RV) dysfunction predicts mortality in COVID-19 patients, which is frequently secondary to acute respiratory distress syndrome [57]. Li et al. performed echocardiographic examination in 120 COVID-19 patients and measured the conventional RV functional parameters including RV fractional area change, TAPSE (tricuspid annular plane systolic excursion), and tricuspid tissue Doppler annular velocity, as well as RVLS (right ventricular longitudinal strain) obtained by speck-

le-tracking echocardiography. In fact, it has been recently demonstrated that RVLS is a more accurate tool to estimate RV function. It was found that non-survivors had enlarged right cardiac chambers, declined RV function, and elevated pulmonary artery systolic pressure compared to survivors. Additionally, RVLS, RV fractional area change, and TAPSE were significant univariate predictors of a higher mortality risk ($p < 0.05$). Patients in the lowest RVLS tertile, in comparison with those in the highest tertile, were more likely to have higher heart rates, as well as elevated D-dimer and CRP levels. They also required more high-flow oxygen and invasive therapy. The incidence of acute myocardial injury, acute respiratory distress syndrome, and deep-vein thrombosis was higher in the lowest tertile. Furthermore, the same association was observed in the case of mortality. The best cut-off value of RVLS for prediction of fatal outcomes was −23% (AUC: 0.87; $p < 0.001$; sensitivity, 94.4%; specificity, 64.7%). The abovementioned results support the application of RVLS in the risk stratification in COVID-19 patients [61].

CMR with its new quantitative mapping techniques is particularly effective in the diagnosis and risk stratification of acute myocardial injury in the course of COVID-19. A systematic review of 34 studies comprising CMRs in 199 patients revealed that only 21% of examinations were with the normal limits. The most prevalent diagnosis was myocarditis (40.2%) (presented in **Figure 3**). Furthermore, T1 (109/50; 73%) and T2 (91/144; 63%) mapping abnormalities, edema (46/90; 51%) and late gadolinium enhancement (85/199; 43%) were the most common findings. Late gadolinium enhancement was most commonly seen in the subepicardial location (81%) in inferior segments [62]. Interestingly, it was present in a lower proportion in COVID-19 subjects when compared to patients with myocarditis, yet without COVID-19. This supports the hypothesis that inflammation constitutes the primary mechanism of myocardial injury in SARS-CoV-2 infection [63].

CT allows for the evaluation of lung parenchyma, patency of coronary and pulmonary arteries, and the assessment of myocardial injury, which renders it a comprehensive, non-invasive imaging modality allowing "quadruple rule-out" of most serious CV complications in the course of COVID-19 infection [64]. Although CMR is more frequently used in myocarditis detection, imaging of myocardial fibrosis by CT is feasible. Specifically, late iodine enhancement CT and extracellular volume CT are able to identify focal fibrosis and diffuse myocardial injury, respectively [65]. Furthermore, CT coronary angiogram can replace invasive coronary angiography in excluding obstructive CAD for patients with thoracic pain in the course of infection. In addition, it also allows for the estimation of the already discussed CCS, which is an independent predictor of in-hospital mortality and ICU admission [21]. Finally, CT pulmonary angiography has a crucial role in the diagnostic evaluation of COVID-19 complicated with acute PE [64].

Summary

Patients suffering from COVID-19 are at risk of developing CV complications and require close monitoring, especially those with CV comorbidities.

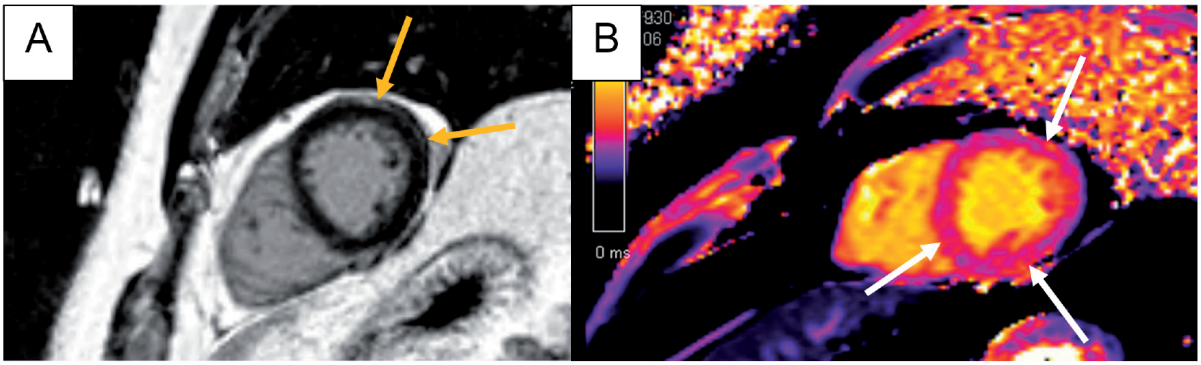

Figure 3. Myocardial injury on CMR 3 months after COVID-19: A: intramural fibrosis – late gadolinium enhancement in short axis view (arrows); B: diffuse fibrosis – T1 map in short axis view; native T1 in affected area = 1029ms (normal values: 967 ± 14 ms) [with the courtesy of Prof. Małgorzata Pyda; Poznan University of Medical Sciences CMR Unit, Cath Lab]

SARS-CoV-2 enters cardiomyocytes via their ACE2 receptor and interferes with its cardio-protective activity in the process. The systemic inflammation caused by COVID-19 further damages the heart by mis-balancing electrolyte levels and the myocardial demand-supply ratio. Decreased blood oxygen levels from pulmonary damage subsequently contribute to complications. As a result, the negative impact of SARS-CoV-2 on the cardiovascular system may manifest as myocardial injury, myocarditis, AMI, HF, arrhythmias, and venous thromboembolic events. In addition, some of the medications used to combat the disease, have a negative impact on the CV properties.

We analyzed numerous CV factors in order to identify those indicating an increased probability of a fatal outcome. As SARS-CoV-2 disproportionately affects people suffering from CV diseases, a prior CV disorder (especially hypertension, CAD and HF) is the greatest risk factor of unfavorable prognosis. Similarly, the development of any serious CV complication in the course of COVID-19 decreases the chances of survival.

Fortunately, there are certain risk factors which can also contribute to an increased alertness in terms of the anticipated disturbing symptoms and better prevention. The use of the CCS in addition to the high-resolution CT deserves special attention, since it helps in identifying patients with CAD with a higher baseline risk of adverse outcomes without additional costs. Another example is high D-dimer levels, which are vital in the process of ruling out thrombotic events. Markedly worse outcomes of COVID-19 patients with thrombotic complications highlighted the importance of thrombosis prophylaxis in all subjects admitted to ICUs. Furthermore, as the development of new-onset arrhythmia is associated with a severe disease course, continuous telemetry constitutes another useful tool in COVID-19 management. In addition, extra caution should be taken in patients with inherited arrhythmic disorders due to their increased risk of developing life-threatening arrhythmic events. ECG abnormalities during hospitalization also correlate with a higher risk of major adverse events.

Cardiac biomarkers can be used to assess the severity of the SARS-CoV-2 impact on the CV system. The elevated levels of TnI indicating myocardial necrosis have been associated with an increased risk of mortality, and have been observed even in patients without ACS Myoglobin and CK-MB have also been suggested as possible negative prognosis predictors regarding in-hospital death and non-recovery. Furthermore, NT-proBNP has been independently correlated with in-hospital death following adjustment for potential risk factors. In turn, the role of lactate dehydrogenase is uncertain, as elevated levels reflect multiple organ injuries. Nevertheless, it may play a prominent role in the triage of patients with COVID-19.

Overlapping clinical presentations and complex etiology of myocardial injury in COVID-19 require additional cardiac imaging to establish the diagnosis, guide therapy and stratify the risk of fatal outcome. Bedside ultrasound assessment of the heart and vessels is an effective first-line tool in detecting any abnormalities, although some patients may need advanced techniques. It is postulated that RV dysfunction predicts mortality in COVID-19 patients, and RVLS is a particularly accurate parameter. Furthermore, CMR is especially advantageous for the diagnosis of myocarditis. Finally, CT is efficient in the evaluation of lung parenchyma, patency of coronary and pulmonary arteries, and myocardial injury, making it the best non-invasive imaging modality allowing "quadruple rule-out" of the most serious CV complications.

We believe that the negative impact of SARS-CoV-2 not only on the respiratory, but also on the circulatory system, requires an integrated assessment of numerous CV risk factors of poor prognosis. Clinical evaluation of the possible CV complications, laboratory tests, ECG-monitoring, and CV imaging should be applied in order to provide the best possible management to COVID-19 patients, particularly those requiring hospitalization.

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

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IMAGES IN CLINICAL MEDICINE

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Acute Aortic Thrombus with Splenic Infarction in a Patient with COVID-19 Infection

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ABSTRACT

Acute Aortic thrombus with splenic infarction is a rare complication of COVID-19. This manuscript highlights the importance of early identification of this complication with abdominal imaging and early initiation of anticoagulation despite moderate severity of the disease.

Description

A 40-year male with COVID-19 infection presented to the emergency department of a dedicated COVID-19 hospital with complaints of dyspnoea and fever of 2 days duration. His nasopharyngeal and oropharyngeal swabs were positive for COVID-19, and he had been under home isolation for 5 days. He had already received azithromycin 500 mg OD and Ivermectin 12 mg OD for 3 days, doxycycline 100 mg BD for the next 2 days, and analgesics for 5 days. On admission, his oxygen saturation was 93% on room air, heart rate 96/min, blood pressure 136/86 mm Hg, the temperature of 39.1°C, a respiratory rate of 21–25. He did not have any comorbidities. High-resolution computed tomography (CT) chest (**Figure 1**) demonstrated multiple peripheral areas of consolidation and ground-glass opacities significant of viral pneumonitis. His CT severity score (CTSS) was 9/25. Laboratory test revealed mildly increased CRP (0.8 mg/dL), ferritin (668 ng/dL), LDH (378 U/L), and normal D-dimer. The patient was kept on supplemental oxygen and treated with methylprednisolone 20 mg IV BD and ceftriaxone 1 gm OD. On day 4 of his admission, he developed severe acute abdominal pain in the left hypochondrium. On examination, his abdomen was soft, non-tender, and he presented no organomegaly on palpation. An emergency abdominal contrast-enhanced CT (**Figure 2**) was performed which revealed a thrombus in the upper abdominal aorta and splenic artery with the associated partial splenic infarction. There was no sign of aortic atherosclerosis. Electrocardiogram and Echocardiographic examinations were normal. His coagulation profile revealed elevated D-dimer (1800 ng/ml) with mildly decreased plate-

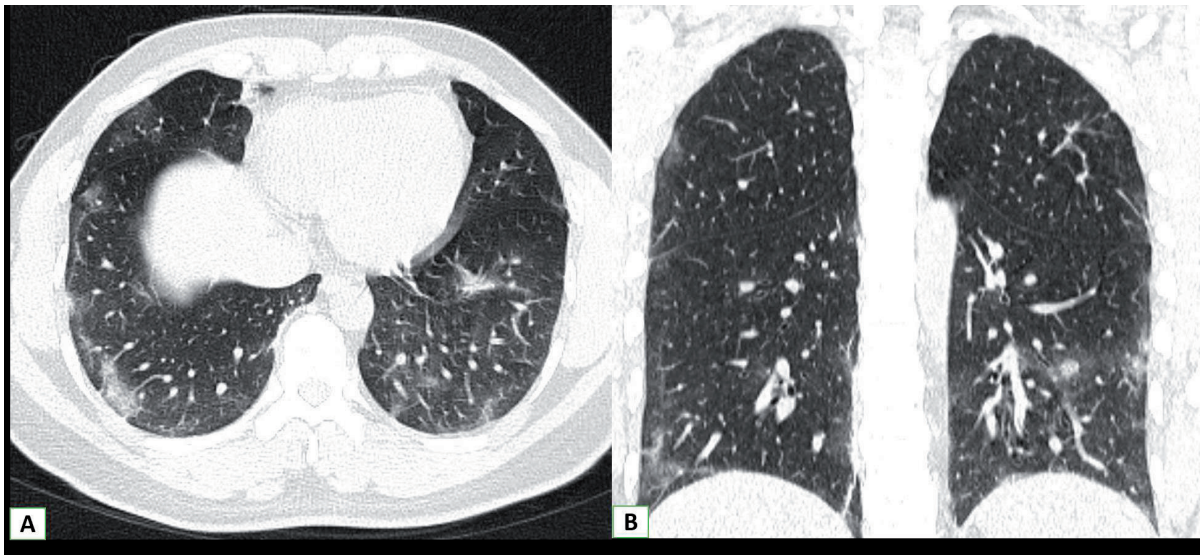

Figure 1. High resolution computed tomography of the thorax, axial (A) and coronal (B) images present multiple patchy areas of ground-glass opacities and consolidation in bilateral lungs predominately in the subpleural location. Findings are consistent with COVID-19 pneumonia

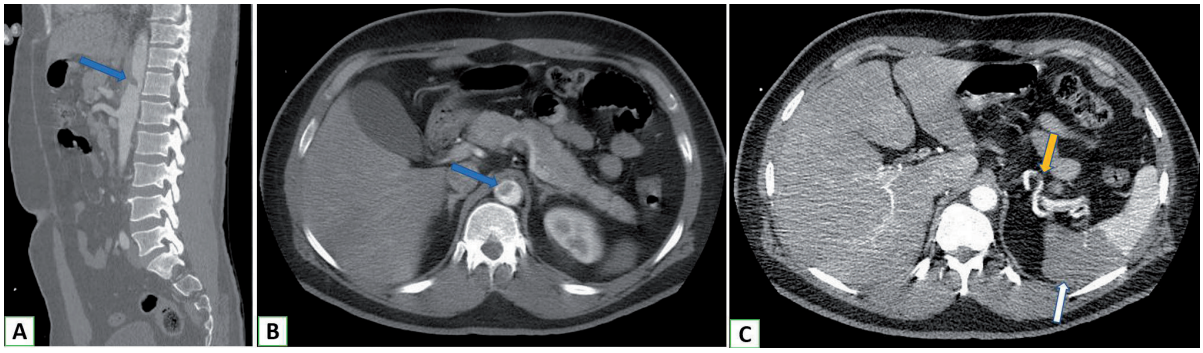

Figure 2. Contrast-enhanced computed tomography (CECT) of the abdomen; sagittal (A) and axial (B and C) images show a small non-enhancing filling defect in the abdominal aorta (blue arrows) and splenic artery (yellow arrow) significant of thrombus. Associated partial splenic infarction is presented in picture C (white arrow)

let count (100 x 10^3/µl). As the aortic thrombosis was small, partial and non-obstructive on CT, the patient was scheduled for initial therapeutic anticoagulation, followed by catheter guided thrombolysis if the thrombus persisted. The patient was treated with tramadol and heparin bolus (80 units/kg), which were followed by a continuous infusion of heparin (18 units/kg/hr) for 24 hours. The patient responded positively, and a complete resolution of abdominal symptoms and thrombosis was observed. Subsequently, Low Molecular Weight Heparin (LMWH) 60 mg twice daily was administered for 5 days (the patient's weight was 64 kg and serum creatinine amounted to 1.6 mg/dl). Doses of steroids were gradually reduced and discontinued, whereas the anticoagulant medication was replaced with oral rivaroxaban (10 mg) OD at discharge. At discharge (11th day of his admission), the patient was clinically asymptomatic, his oxygen saturation returned to 96% at room air and he tested negative in the real-time PCR assay.

Thrombotic events are currently recognized as one of the major complications of COVID-19 infection. In fact, a hypercoagulable state in COVID-19 patients predisposes them to both arterial and venous thrombosis [1]. The proposed mechanism for thrombosis includes endothelial damage, pro-inflammatory cytokine release, systemic inflammatory response, hypoxia, and

disseminated intravascular coagulation [1]. Furthermore, the presence of thrombotic complications has also been positively correlated with the severity of the disease [2]. Additionally, solid-organ infarction develops secondary to thrombosis of the vessel supplying the organ; however, in most cases, no thrombus is visible possibly due to the presence of multiple microthrombi [3]. Splenic infarctions are usually treated conservatively, unless some complications or exacerbations of symptoms occur. Thus, acute aortic thrombus can be treated either with therapeutic anticoagulation, catheter guided thrombolysis, or surgical thrombectomy depending on the clinical symptoms, as well as on the extent and size of the thrombus [1]. Thrombotic events may constitute the presenting symptoms of COVID-19 infection, or they can develop subsequently in the course of the disease. This, in turn, highlights the significance of abdominal imaging in patients reporting abdominal symptoms [3]. Moreover, in cases presenting solely with abdominal symptoms, imaging examinations of the abdomen allow for the identification of COVID-19 lesions in lung bases, hence providing a clue with regard to the diagnosis [4]. Our case further validates the employment of anticoagulant prevention in all patients with moderate to severe course of the disease, despite the absence of comorbidities, and initial normal D-dimer as well as coagulation profile.

Learning Points

1. COVID-19 infection is a prothrombotic state with a high risk of both arterial and venous thrombosis.
2. COVID-19 patients presenting with abdominal symptoms should undergo abdominal imaging examinations aimed at ruling out thrombotic complications.
3. Splenic infarction is a rare disorder which can present with left-sided abdominal pain and can be secondary to a hypercoagulable state in COVID-19 patients.

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Contributors
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Conflict of interest statement
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App-assured essential physical activity for the prevention of cognitive decline: changing paradigms in public health – a study protocol for a randomised controlled trial

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ABSTRACT

This study aims to assess the effect of an increase in daily physical activity to prevent cognitive decline, sustain brain volumes and maintain healthy biomarker levels in mild cognitive impairment (MCI) subjects

aged 50-70 years. In total, 198 subjects with MCI (assessed using the Montreal Cognitive Assessment test) will be recruited and randomised into two groups: active and passive. The active group will be instructed, encouraged and motivated to increase their physical activity to at least a moderate level (≥ 10,000 steps/day), whereas the passive group should maintain their normal activity levels. All subjects will undergo cognitive assessment, neuroimaging and biomarker tests prior to and after a one-year intervention. During the intervention, physical activity will be measured by the Fitbit Inspire HR wristband. The study was registered in the German Clinical Trials Register database (registration no. DRKS00020943, date of registration: 09.03.2020, protocol version: 1.0).

Research Project Objectives

The study aims to assess the effect of increasing daily physical activity on the prevention of cognitive decline, sustaining brain volumes, as well as on maintaining healthy biomarker levels in subjects aged 50–70 years affected by mild cognitive impairment (MCI). The study hypotheses are as follows:

1. Increased daily physical activity to at least a moderate level (> 10,000 steps/day) for one year will not affect cognitive function.
2. Higher levels of daily physical activity (> 10,000 steps/day) will not preserve brain volume and will not maintain proper values of healthy biochemical markers and anthropometric parameters.

Research Plan and Basic Concept

Basic Concept

MCI is a condition in which subjects demonstrate cognitive decline with minimal dysfunction of instrumental daily activities, which may also be a stage preceding dementia [1]. According to a recent systematic review, about 18% of MCI subjects develop dementia within two years, with the conversion rate increasing to 32% following five years [2]. In 2016, the global prevalence of dementia was 48.3 million [3], which is anticipated to increase to 80 million in 2030 [4]. Neurocognitive disorders significantly affect everyday living and place a substantial financial burden on healthcare systems. Advanced age and family history of neurocognitive disorders are important risk factors for developing dementia, as well as numerous modifiable risk factors, such as hypercholesterolaemia, hypertension, obesity, hyperglycaemia, poor education and physical inactivity [5]. Moreover, currently, there is no pharmacological treatment approved for MCI. Therefore, it is crucial to identify MCI subjects and attempt to mitigate the risk factors in this group [6].

To date, there have been several studies regarding the impact of physical activity on the prevention of cognitive decline [7–9]. A recent meta-analysis demonstrated that physical activity (aerobic, resistance training or tai chi) positively affects cognitive function in adults aged 50 years or older, regardless of their baseline cognitive status [7]. In addition, another meta-analysis showed that slow walking and jogging significantly improved attention, execution and memory processes [8]. Furthermore, improved daily physical activity, defined as walking a greater distance, helps to preserve grey matter volume in the frontal, occipital, entorhinal, and hippocampal regions, resulting in a reduced risk of cognitive decline [9, 10].

Although several studies reported a relationship between physical activity and cognitive functions preservation, there is no consensus regarding the exact frequency, duration, intensity and type of exercise necessary to prevent cognitive decline. Current physical activity guidelines recommend that adults should be involved in at least 150 minutes of moderate-intensity aerobic exercise a week [4, 11]. However, only less than 5% of adults were able to comply with the recommendations which indicates that the existing guidelines are too demanding for the elderly. Moreover, actual physical activity was lower compared to the declared level of activity in all forms of questionnaires [12].

Study design

The study was designed as a parallel-group prospective randomised controlled trial. The study protocol was registered in the German Clini-

cal Trials Register database (registration no. DRKS00020943, date of registration: 09.03.2020, protocol version: 1.0). The study protocol is reported in accordance with the Standard Protocol Items: Recommendations for Interventional Trials guidelines [13, 14]. The data in this study will be reported and presented according to the Consolidated Standards of Reporting Trials statement [15].

Ethical issues

The present study will be conducted according to the guidelines provided in the Declaration of Helsinki. The study protocol was approved by the Bioethics Committee of Poznan University of Medical Sciences (refs. 47/20, 169/20, 263/20, 481/20, 720/20, 296/21 and 555/21). The study personnel will obtain written informed consent from all study participants upon their enrolment. The study will not have a data monitoring committee, given that we do not anticipate severe adverse effects. Study-related personal damage of the participating subjects will be covered by the Poznan University of Medical Sciences insurance policy. Should the study protocol require amendments, a revised version will be submitted to the Bioethics Committee. The study team and the study participants will be also informed about all the changes.

Study population

In total, 198 subjects will be recruited to the study. Inclusion criteria are as follows: age 50–70 years, diagnosed MCI (the result of the Montreal Cognitive Assessment (MoCA) test: 19–26 points), community residence, and owning a smartphone. On the other hand, the exclusion criteria include: depression and/or the results of the Hamilton Depression Rating Scale (HAM-D) > 13 points, use of cognitive boosting medications or psychotropic medications, substance abuse disorders (e.g. alcohol > 15 drinks (units)/week), diagnosed psychiatric disorders, Parkinson's disease, Alzheimer's disease, dementia, anaemia, diabetes of at least 10 years, chronic renal and liver diseases, a history of cancer within the past five years, history of stroke, current evidence or a history of seizures in the past two years, head injury with loss of consciousness and/or immediate confusion following the injury, hypothyroidism with current misaligned thyrotrophic hormone levels, any chronic diseases which limit training and testing of cardiovascular and respiratory systems, current intensive physical activity (at least 10,000 steps/day), implanted pacemaker, neurostimulator and other metal components, including prosthetic implants, blindness, deafness, language difficulties or any other disability which may prevent subjects from participating, or cooperating in the protocol.

Recruitment

Participants will be recruited to the study from patients of medical clinics and medical centres in the Greater Poland region (Poland) in consultation with their physicians and directors of the clinics, by means of study promotion via workplace channels at the university and healthcare services, as well as via university newsletters and websites, posters, leaflets and email invitations sent to companies, offices, and institutions for distribution to their employees. The research team will contact the interested participants and send further information about the study. Prior to the commencement of the study, the potential subjects will be screened by a physician during an inclusion appointment to comply with the protocol requirements. In this phase, cognitive functions will be evaluated by the MoCA test and the HAM-D scale will be used to assess the occurrence of depression symptoms. Additionally, physical activity will be determined for at least one week before the enrolment using the Fitbit Inspire HR tracker. Subjects will receive information regarding the study, its purpose, putative benefits, and the possible risks. All subjects will be informed that participation in that study is voluntary, and that they may refuse to participate, or withdraw from the trial at any time without providing reasons.

Intervention

The study population will be randomised (allocation ratio: 1:1) into two groups: active (group A) or passive (group P). Group P (n = 99) will be asked to sustain their normal activity, whereas group A (n = 99) will be asked, instructed and motivated by the mobile application to increase their physical activity intensity to at least 10,000 steps/day. During the intervention period, all subjects will be instructed to maintain their current diet and

maintain their medications and, if they change, to record this in a diary. Prior to and after the one-year intervention period, cognitive functions, neuroimaging, and biochemical parameters will be assessed in all study subjects. Additionally, physical activity will be determined using the Fitbit Inspire HR tracker. Moreover, before, during and after the intervention period, anthropometric and densitometric parameters, body composition, as well as dietary habits will be assessed. A self-administered questionnaire regarding physical activity, health condition, medications, smoking, alcohol use, profession, and education will be distributed to subjects. Basic clinical examinations and measurements will also be performed. The scheme of this study is presented in **Figure 1**.

Adherence to the intervention

Adherence to the intervention will be assessed by data collected from the Fitbit, including the number of steps per day, distance travelled, estimated energy expenditure, sedentary behaviour, minutes of low, moderate and intensive activity, as well as sleep behaviour. The data will be wirelessly uploaded to the user's account and will

	STUDY PERIOD			
	Enrolment	Allocation	Post-allocation	Close-out
TIMEPOINT	$-t_1$	t_0	t_1 (6th month)	t_2 (12th month)
ENROLMENT:				
Eligibility screen	x			
Informed consent	x			
Medical examination	x			
Hamilton Depression Rating Scale	x			
Montreal Cognitive Assessment	x			
Physical activity (Fitbit)	x			
Allocation		x		
INTERVENTIONS:				
Active group (> 10 000 steps/day)		◆———	———	———◆
Passive group (< 10 000 steps/day)		◆———	———	———◆
ASSESSMENTS:				
Primary outcomes:				
Montreal Cognitive Assessment	x			x
Cambridge Neuropsychological Test Automated Battery		x		x
Secondary outcomes:				
Anthropometric parameters (body high, body weight, waist and hip circumferences, body mass index)		x		x
Biochemical markers (fasting glucose and insulin homeostasis markers, lipid profile, inflammatory markers, markers of neuronal growth and destruction)		x		x
Blood pressure		x		x
Body composition and densitometric parameters (DEXA)		x		x
Food Frequency Questionnaire		x	x	
Hamilton Depression Rating Scale	x			x
International Physical Activity Questionnaire		x	x	x
Magnetic resonance imaging		x		x
Physical activity (Fitbit)		x	x	x
Socioeconomic assessment		x		x
3-day dietary record		x		x

Figure 1. The study schedule of the enrolment, interventions, and assessments

be downloaded by our research team through the Fitbit website, or by means of the application programming interface. The Fitbit data will provide us with objective information regarding the level of adherence throughout the intervention period. Furthermore, in order to increase adherence to the intervention, phone calls will be scheduled to review the compliance with the physical activity guidelines, and all participants will also be given the option of additional calls if necessary. In addition, weekly emails, including the information about their average step count, will be sent to the study participants. Moreover, a check-up appointment will be conducted six months after starting the intervention in order to verify the subject's adherence to the intervention. The study participants who will not comply with the intervention will be excluded from the study, and the principal investigator will make a final decision regarding the exclusion. If a participant decides to withdraw from the study, no further data will be collected concerning this individual.

Minimum sample size calculation

The minimum sample size was calculated on the basis of a recent physical activity intervention study in subjects with an incident of cognitive impairment. It demonstrated that the executive function and memory scores were -0.33 ± 0.79 and -0.32 ± 1.29, in the low active and 0.31 ± 0.86, and 0.22 ± 1.05 in the highly active group [16]. However, we assume that due to the preselection, we will manage to obtain a more homogenous group, and thus achieve a more significant clinical effect and 1) the probability of a type-I error at an alpha cut-off level of 5% ($\alpha = 0.05$); 2) the probability of a type-II error at a beta cut-off level of 20% ($\beta = 0.2$); 3) the difference of the anticipated means equals to 0.62 standard deviation (SD); 4) the expected value of SD equals to 85% of the mean.

Randomisation and blinding

Randomisation will be performed via computer software (RRApp Robust Randomization App, the Icahn School of Medicine at Mount Sinai, New York, NY, USA [17]) and the data will be uploaded by an independent researcher. We will perform blocked randomisation (block size: six) with the stratification according to sex and prevalence of diabetes. The participants will be allocated in equal numbers to one of two groups, passive or active, as defined by the code. According to the character of the intervention, the study participants and researchers taking the physical activity measurements will not be blinded to the allocation. Only the outcome assessors and the study team members who will prepare the database and will perform the statistical analysis will be blinded.

Protection of data privacy

Quantitative data will be collected from the recruited subjects using anthropometric, clinical, biochemical and behavioural measurements to written and electronic files, and subsequently to permanent file formats for analysis. Subjects will be identified by non-personal codes and tied to metafiles. The data will be verified by investigators. Original written documents will be stored in a locked filing cabinet, whereas all the data will be collected in secure access computers. Documents and files will be retained as authorised by the Bioethics Committee. The final trial dataset will be accessed by the principal investigator, study coordinator and other team members.

Dissemination

The study results will be presented at local, national and international conferences, and will be published in open-access peer-reviewed journals. Authorship eligibility will be based on the International Committee of Medical Journal Editors. The data collected in this study will be available on request from the principal investigator. Study participants will be informed of the outcomes of the study.

Research Methodology

Primary and secondary outcomes

The primary outcomes of the study will be changes (Δ before – after) in cognitive function parameters assessed by the MoCA test and the Cambridge Neuropsychological Test Automated Battery (CANTAB), whereas the changes in biochemical parameters, neuroimaging, anthropometric parameters, body compositions and densitometric parameters will be regarded as the secondary outcomes. All the data, except neuroimaging, will

be collected in the Department of Pediatric Gastroenterology and Metabolic Diseases, Poznan University of Medical Sciences. Brain magnetic resonance imaging (MRI) will be performed at the Heliodor Swiecicki Clinical Hospital in Poznan. Blood samples will be collected by a commercial laboratory, while the biochemical parameters will be measured at the Laboratory of the Department of Pediatric Gastroenterology and Metabolic Diseases, Poznan University of Medical Sciences, or by a commercial laboratory.

Anthropometric measurements

Basic anthropometric parameters (body height, body weight, hip and waist circumferences) will be measured before and after the intervention period. Body mass index will be calculated on the basis of body weight and body height measurements. During the anthropometric measurements, all participants will wear light clothes and will be barefoot, with an average of two measurements recorded.

Body composition and densitometric parameters

Before and after the intervention period, body composition (fat and free fat mass), bone mineral density and the content of the total body and lumbar spine (L1-L4) will be assessed by means of dual-energy X-ray absorptiometry methods using the Hologic Discovery analyser (Bedford, Massachusetts, USA).

Blood pressure

Blood pressure will be measured prior to blood sample collection according to the guidelines of the European Society of Hypertension. Blood pressure will be measured on the arm at the heart level, and will be expressed by three measurements of the systolic and diastolic pressure [18].

Assessment of dietary habits

Dietary habits will be assessed before and during the intervention period by means of 3-day dietary records covering two weekdays and one weekend day. Participants will be asked to give a detailed description of foods consumed and to estimate their quantity. To investigate food group intake, the Beliefs and Eating Habits Questionnaire created by the Behavioural Conditions of Nutrition Team, Committee of Human Nutrition Science of the Polish Academy of Sciences will be administered [19]. The energy intake and the basic nutritional compounds (carbohydrates, proteins, and fats), selected vitamins and minerals, dietary fibre, cholesterol, saturated, monounsaturated and polyunsaturated fatty acids intake will be assessed using the Aliant software (Anmarsoft, Gdańsk, Poland). The nutrition standards for the Polish population will be applied to determine whether individual dietary intakes meet the nutritional recommendations [20].

Physical activity

Physical activity will be determined using the Fitbit Inspire HR tracker (Fitbit Inc., San Francisco, USA). In addition, the International Physical Activity Questionnaire will be used to assess physical activity before, during and after the intervention period.

Fitbit Inspire HR is a wrist-worn wearable wireless sensor with an accelerometer recording physical activity throughout the day, which can synchronise with a smartphone application and a computer. Therefore, participants will be instructed to download the Fitbit app, and will be asked to wear the Fitbit all day, except when showering, bathing, and swimming. Participants will be instructed to wear the Fitbit on their non-dominant wrist for one year. In general, the Fitbit requires the creation of individual user accounts to download the stored data using a Web-based software application. Nevertheless, for the purpose of this study, user accounts will be created by the study team which can only be accessed by the researchers. Physical activity data will be stored on the individual accounts of study participants and will be downloaded of each participant's wearing period by the study team.

Hamilton depression rating scale

The HAM-D scale was used during the inclusion visit and after the intervention to assess the prevalence of depression symptoms [21]. The scale predominantly assesses cognitive and vegetative symptoms, with relatively few items related to social, motor, anxiety and mood factors. The 17-item HAM-D was employed in the present study, each item is scored from 0 to 2 or from 0 to 4, with total scores ranging from 0 to 52. The following cut-off points were used: ≥ 23 – very severe depression, 18–22 – severe depres-

sion, 14–18 – moderate depression 8–13 – mild depression and < 7 – not depressed [22].

Cognitive assessments

The CANTAB and MoCA tests will be employed in this study as the primary outcome parameters. The included tests comprise the following categories: executive functioning, processing speed, memory and abbreviated memory. The following test batteries will be involved in the present study: Motor Screening Task, Reaction Time, Paired Associates Learning, Spatial Working Memory, Pattern Recognition Memory, Delayed Matching to Sample, Rapid Visual Information Processing.

Neuroimaging protocol

Brain magnetic resonance imaging will be performed on all subjects using a Siemens Skyra 3T magnetic resonance imaging (MRI) System. The following MRI sequences will be used in every examination: 1) T2-weighted (fast-spin echo) and modified T2-weighted fluid-attenuated inversion recovery sequence for the detection and localisation of ischaemic lesions; 2) diffusion-weighted with an apparent diffusion coefficient map for the detection of acute ischaemic focuses; 3) susceptibility-weighted imaging for identification of intracerebral haemorrhagic and microhaemorrhagic lesions; 4) 3D angiographic time of flight sequence for visualisation of blood flow in intracerebral arterial vessels; 5) T1-weighted 3D spoiled gradient-recalled echo sequence (3D volumetric sequence) for estimation of total and segmented brain volume.

Blood collection and biochemical analysis

Blood samples will be collected from the antecubital vein via standard venepuncture performed by registered staff nurses. The samples will be taken from the participants after 12-h fasting. The following blood biomarkers will be measured: fasting glucose and insulin homeostasis markers, lipid profile, inflammatory markers (interleukin 6, interleukin 1 receptor type alpha, tumour necrosis factor-alpha, high-sensitivity C-reactive protein), as well as neuronal growth and destruction markers (brain-derived neurotrophic factor, amyloid β-40, amyloid β-42 ratio and phosphorylated Tau protein). Other biochemical and genetic analyses are planned as optional if further funding is available.

Sociodemographic and medical history questionnaires

Background, place of residence, education, family status, and economic status will be assessed before and after the intervention using a sociodemographic questionnaire. The participants will also answer questions regarding lifestyle factors, including tobacco smoking habits and alcohol consumption. A medical history questionnaire will be used to assess the health status of the study participants and to verify whether the subjects receive any medications or dietary supplements.

Statistical analyses

The STATISTICA (StatSoft, Tulsa, USA) software, or equivalent, will be used for the statistical analysis. A two-sided p-value < 0.05 will be considered statistically significant. The overall characteristics of subjects will be expressed as a mean and SD with 95% confidence interval, median and interquartile range, or as frequencies and percentages. The outcomes will also be expressed as changes between the post- and pre-intervention values (Δ value at 1 year). The normality of the variable distribution will be verified on the basis of the Shapiro-Wilk normality test. Comparisons between two unpaired groups will be determined using t-tests or Mann-Whitney U tests, respectively. The Wilcoxon test will be used to analyse the statistical significance of the pre- and post-intervention variables. The above non-parametric tests will be used, if the data either do not conform to normality or cannot be normalised by log-transformation. Otherwise, an analysis of covariance will be used to compare the differences between two groups with the baseline data as the covariate and the potential confounders added to the model. Contingency tables will be used to assess relationships between the categorical variables. Depending on the data distribution, parametric (Pearson's) or nonparametric tests (Spearman's) will be applied to assess correlations. Uni- and multivariate logistic and linear regression analyses will be used to identify independent determinants of cognitive functions. Potentially confounding factors from these univariate analyses will subsequently be entered in a multivariate linear regression analysis. In a stepwise multivariate analysis, factors

for inclusion will be set at $p < 0.1$. In terms of the categorical variables, dummy variables should be entered in the linear regression analysis. If any data are missing, we will assume that they all follow a multivariate normal distribution and adopt multiple imputation approaches. There are no planned interim statistical analyses, or formal stopping rules with regard to efficacy. For the main-outcome parameters, a correction for multiple testing will be applied, unless a multivariate model can be used which produces one single test.

Measurable Effects

This study will potentially provide additional information which allows a more efficient and precise planning of daily physical activity for MCI subjects. We expect that the study will produce exact values for the physical activity intensity required to protect against cognitive decline. The study findings might also be useful for developing first physical activity guidelines aiming to protect against cognitive impairment.

Expected Results

In the proposed randomised controlled trial, a 12-month physical activity intervention will be performed in a group of 198 subjects with MCI and aged 50–70 years. On the basis of both the current literature and new findings, we aim to establish thresholds of the intensity and frequency of physical activity which will serve to develop novel physical activity guidelines to protect against cognitive decline in high-risk adults. We will also investigate associations between physical activity, cognitive function, brain volume, and blood biomarkers. We assume that there are thresholds of physical activity frequency and intensity, which improve global cognitive functions in at-risk individuals, preserving brain volumes and maintaining biomarker levels within the normal limits. The expected findings will allow us to develop the first specific, cognitive impairment-focused physical activity guidelines, which will be effective and achievable for older subjects. Walking, as a form of physical activity, is inexpensive, easy to perform and protects from other chronic diseases, such as diabetes, cardiovascular diseases, obesity, and depression. Therefore, a simple physical activity tracker with a mobile application could be a helpful tool in increasing compliance.

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M.J., A.M. and M.W.G. wrote the manuscript. J.B., H.W.W. and Z.P. commented on the manuscript, J.K.N., K.-H.H. and E.M. designed the study and edited the manuscript. J.W. designed the study and commented on the manuscript, as well as supervises and coordinates the study. All authors read and approved the final manuscript.

Conflict of interest statement

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