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REVIEW

Physiopathological mechanisms related to inflammation in obesity and type 2 diabetes mellitus

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Abstract

Overweight, obesity, and type 2 diabetes mellitus pose global health problems that are ever-increasing. A chronic low-grade inflammatory status and the presence of various pro-inflammatory markers either in circulation or within dysfunctional metabolic tissues are well established. The presence of these factors can, to some extent, predict disease development and progression. A central role is played by the presence of dysfunctional adipose tissue, liver dysfunction, and skeletal muscle dysfunction, which collectively contribute to the increased circulatory levels of proinflammatory factors. Weight loss and classical metabolic interventions achieve a decrease in many of these factors' circulating levels, implying that a better understanding of the processes or even the modulation of inflammation may alleviate these diseases. This review suggests that inflammation plays a significant role in the development and progression of these conditions and that measuring inflammatory markers may be useful for assessing disease risk and development of future treatment methods.

Key Words: Adipose tissue; Biomarkers; Diabetes; Inflammation; Obesity pathophysiology

Core Tip: A significant amount of literature indicates the relationship between increased inflammatory markers and overweight, obesity, and type 2 diabetes mellitus. Even though the role of inflammation in the development and progression of these conditions is uncertain, the potential use of inflammatory markers as diagnostic and prognostic tools is under vigorous investigation. Weight loss and lifestyle interventions result on reduction of inflammatory markers in individuals with overweight and obesity and/or type 2 diabetes mellitus.

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INTRODUCTION

Overweight and obesity represent a significant, ever-increasing global public health challenge[1-4]. Obesity is a complex multifactorial disorder linked to a high risk of developing type 2 diabetes mellitus (T2DM), cardiometabolic diseases and most recently coronavirus disease 2019 (COVID-19)[2,5-10]. Excess and ectopic adiposity and adipose tissue (AT) dysfunction, characterized by a state of low-grade inflammation, underline the pathophysiology of obesity and its consequences to a great extent[8,11-18]. The presence of inflammation in obesity and related metabolic diseases is well established and is proposed to be linked to insulin resistance and/or its further exacerbation, as inflammatory mediators appear to interfere with insulin signal transduction in important metabolic organs (AT, liver, and muscle)[15,19]. Inflammatory markers may be indicators of disease development, allowing us to potentially predict the transition and/or development of complications such as T2DM and cardiovascular diseases[20,21]. Consequently, by achieving an improved understanding of the changes in metabolic and inflammatory processes in these various tissues and organs, and unveiling their properties, we could achieve a better understanding of the pathophysiology of obesity-related complications, including T2DM, and develop better prevention and treatment strategies[22-24]. In this mini-review, we will present evidence of obesity and T2DM-related inflammation, explore the underlying mechanisms in selected tissues and organs, and present potential therapeutic options based on the current literature.

CIRCULATING INFLAMMATORY MARKERS

Systemic inflammation in overweight and obesity

Excess adiposity is related to modestly raised levels of many circulating cytokines and inflammatory factors in both mice and humans; hence, obesity is usually defined as a condition of persistent low-grade systemic inflammation[15,16,25]. Evidence suggests that many of these factors are produced in AT, collectively referred to as adipokines, including hormones (leptin, adiponectin), hormone-cleavage enzymes like dipeptidyl peptidase 4, components or factors regulating the clotting cascade like plasminogen activator inhibitor-1 (PAI-1), and chemoattraction/pro-inflammatory factors including interleukins 1, 6, and 8 (IL-1, -6, -8), tumour necrosis factor alpha (TNF-α), and monocyte chemoattractant protein-1 (MCP-1)[11,22,26-30]. Adipokine expression and/or secretion is altered in states of AT dysfunction and may contribute to obesity-associated diseases[11,26]. Leptin circulating concentrations are elevated in individuals with obesity, and its concentrations are generally strongly and positively correlated with white AT mass[31,32]. At the same time, hypoadiponectinemia is another hallmark of obesity, suggesting a loss of its positive insulin-sensitizing and anti-inflammatory properties[33-35]. In a recent meta-analysis of 60 studies with 45,210 participants, positive correlations with C-reactive protein (CRP), IL-6, and TNF-α were observed for leptin but not for adiponectin, implying an important association between AT hormonal function and inflammatory biomarkers with potentially pathophysiological implications[36]. Moreover, acute-phase proteins, including CRP and serum amyloid A, and white blood cell (WBC) count, are also elevated in obesity and related metabolic diseases[21,29,37-40].

Several genome-wide association studies (GWAS) have been conducted to explore the link between obesity and various conditions, as well as the potential cause-and-effect relationship[41]. These studies have identified over 300 single-nucleotide polymorphisms that are associated with different measures of obesity, such as body mass index (BMI), waist-hip ratio, and other indicators of adiposity[41]. In a large-scale genome-wide association study involving a total of over 40000 individuals of European descent, genetic variants associated with higher BMI were strongly associated with higher high-sensitive CRP levels, indicating a causal relationship between adiposity and inflammation, however the opposite was not recorded[42].

Notably, pro-inflammatory markers were also strongly associated with insulin resistance in most individuals, regardless of the degree of adiposity, implicating either a role or at least a relationship between these molecules and the transition to more insulin-resistant states like T2DM[21,43,44].

Inflammation in T2DM and related cardiovascular complications

Subclinical chronic inflammation appears to be a distinct contributor to the development of T2DM[21,45]. Independent of the initial degree of insulin resistance and obesity, elevated levels of several inflam-

matory biomarkers at baseline in different human populations are predictive of T2DM occurrence[21,45]. Elevated levels of IL-6 and CRP are significantly associated with an increased risk of T2DM[46-48]. An elevated WBC count is also associated with a worsening of insulin sensitivity and predicts the development of T2DM[38]. Increased circulating concentrations of pro-inflammatory cytokines IL-1β, IL-18, TNF-α, (apart from IL-6 and CRP) and low levels of adiponectin are strongly associated with T2DM[49]. Among the markers of blunted fibrinolysis, increased PAI-1 appeared to predict T2DM development independent of insulin resistance and other known risk factors for diabetes[50]. Furthermore, biomarkers indicative of inflammation and endothelial dysfunction, including intercellular adhesion molecule 1 and E-selectin, were positively associated with the incidence of T2DM [51]. Based on these observations, it could also be claimed that these changes may be associated with the various cardiovascular complications often related to T2DM and obesity[52,53].

Finally, a plethora of GWAS has been conducted more recently regarding the association and causality between T2DM and inflammatory biomarkers[54]. Of these IL-1 and IL-6 pathways appeared to be positively associated, however evidence remains elusive[55,56].

PATHOPHYSIOLOGICAL BACKGROUND

As mentioned already, inflammation appears to be linked to insulin resistance and/or its worsening in obesity since it was shown to interfere with insulin signal transduction in critical metabolic organs (AT, liver, and muscle) and potentially contribute to the development of T2DM[15,19,48]. In this section, we will present potential mechanisms driving obesity-related inflammation, primarily in the AT, and the implications of circulatory inflammatory factors on various metabolic and regulatory organs. A summary can be found in Figure 1.

Adipose tissue dysfunction

Excess adiposity, AT dysfunction (characterized by a state of low-grade inflammation), body fat distribution, and ectopic fat deposition, particularly visceral fat deposition, are all central figures in the pathophysiology of obesity and its complications[8,11,14].

Dysfunctional AT is distinguished by adipocyte hypertrophy, which is associated with chronic low-grade inflammation, pro-inflammatory immune cell infiltration, adipokine dysregulation, hormonal resistance, blunted metabolism, reactive oxygen species production, endoplasmic reticulum stress, mitochondrial dysfunction, and altered oxygenation, all of which contribute to ectopic fat accumulation and related complications[13,15,57,58]. The location of lipid storage is a key factor in determining an individual's overall health, as obesity-related complications such as hypertension and the risk of T2DM relate to abdominal (upper body) fat accumulation[14,59-63]. In contrast, fat accumulation in the lower body (gluteofemoral AT) is linked to a lower risk of cardiometabolic disease[59,64-67]. Lower body cell composition, including immune cells, is thought to be primarily associated with enhanced anti-inflammatory properties[17,18]. In accordance with that theory, IL-6 release (as determined by an arteriovenous difference technique model) was much lower in femoral adipose tissue than in matched abdominal tissue[60].

Obesity-related lipid accumulation in non-adipose tissues has significant metabolic effects since it is linked to insulin resistance and, potentially, through molecular mimicry, lipid moieties may trigger inflammatory pathways[11,22,68]. Furthermore, hypertrophic adipocytes are shown to possess a pro-inflammatory phenotype, which may exacerbate insulin resistance, resulting in a vicious cycle[69,70]. Adipocyte and AT inflammation, on the other hand, appears to be required for healthy AT growth and remodeling[71]. That observation implies that inflammation is more than just a harmful process, maybe contributing to AT adaptation to stressors, including excess calorie intake. It is worth noting that drugs used to treat T2DM may reduce inflammation by lowering hyperglycemia. However, these medicines' anti-inflammatory effects are inconsistent, and it is unclear if their favorable metabolic effects are mediated by regulation of chronic low-grade inflammation[72]. Finally, in addition to white AT inflammation in obesity, it appears that brown AT inflammation also exists, at least in animal models of obesity, implicating that dysregulation of this tissue aggregates the obesity-related inflammatory status [73].

Liver

Liver dysfunction linked with obesity, which encompasses the Metabolic Associated Fatty Liver Disease (MAFLD) spectrum, is characterized by complex pathophysiological processes that are currently under vigorous investigation and involve several pathways[74]. It has been postulated that an inability to sufficiently enhance subcutaneous AT triglyceride storage capacity in response to increased caloric consumption and body weight reroutes lipids towards other organs, such as the skeletal muscle and the liver, resulting in ectopic lipid accumulation and lipotoxicity at the cellular level, which leads to insulin resistance (IR) and inflammatory responses in these organs[75-79]. Interestingly, fat molecules appear to serve as ligands for substantial inflammatory signaling pathways in Kupffer cells in the liver and AT macrophages *via* the toll-like receptor 2 and 4 (TLR2/TLR4) signaling cascade[80]. Numerous previous

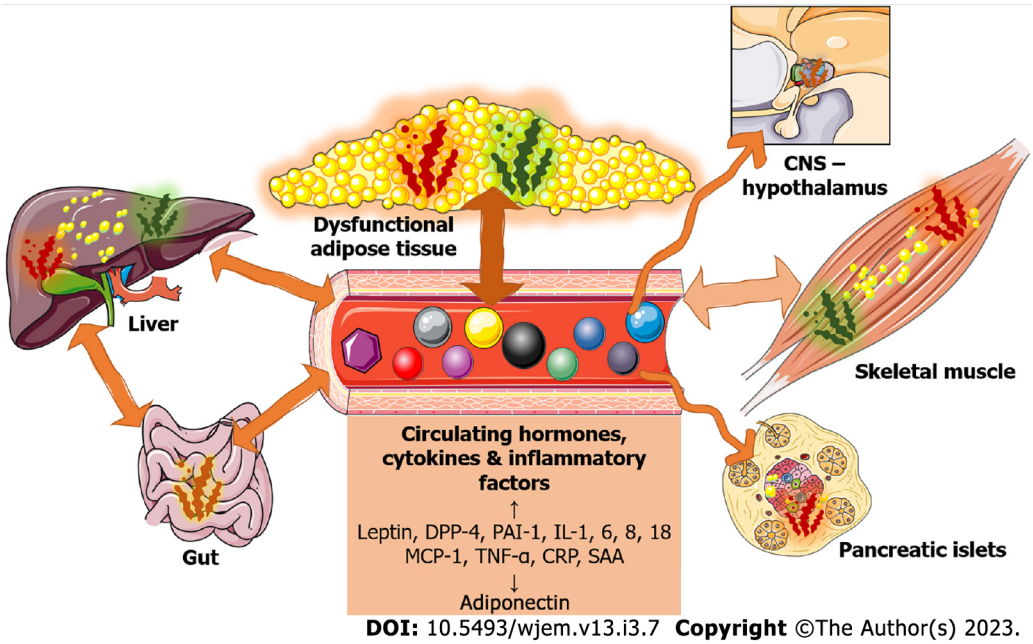

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Figure 1 Potential mechanisms driving obesity-related inflammation, primarily in adipose tissue and other metabolic organs and implications of circulatory inflammatory factors on various metabolic and regulatory organs. CNS: Central nervous system; CRP: C-reactive protein; DPP-4: Dipeptidyl peptidase 4; IL: Interleukins; MCP-1: Monocyte chemoattractant protein-1; SAA: Serum amyloid A; TNF-α: Tumour necrosis factor alpha. Parts of the figure were drawn by using pictures from Servier Medical Art (available from: smart.servier.com).

studies have shown that pro-inflammatory cytokines, specifically TNF- and IL-6, play an important role in the development and progression of NASH[81,82]. TNF- and IL-6 Levels are elevated in the livers and blood of NASH patients, but blocking these mediators improved MAFLD in animal models[81,83].

Muscle

Several pro-inflammatory cytokines have been reported to be overexpressed apart from AT and the liver, also in the skeletal muscle of individuals with obesity and insulin resistance as well as in animal models[84,85]. Obesity progression increases inflammation in skeletal muscle in two ways: Indirectly through AT inflammation and adipocytokines dysregulation, which affect skeletal muscle function and may also augment IR, and directly through ectopic lipid deposition within the skeletal muscle, which initiates several pro-inflammatory pathways[81,86,87]. Myocytes stimulate the production of several hormones and cytokines, collectively called myokines, including IL-6 and IL-15, as well as other molecules like fibroblast growth factor 21 (FGF21) and irisin[75,82]. All these molecules can regulate potential inflammatory processes, and the imbalance of their production in IR, obesity, and T2DM could further aggregate this overall inflammatory status[88]. Sarcopenic obesity, which is more common in older patients, may also cause an increase in unfavorable pro-inflammatory status and impair insulin sensitivity *via* a loss of favorable myokines[9]. Finally, as in the AT and other organs, immune cell infiltration with pro-inflammatory activation in skeletal muscle has been observed, resulting in the release of pro-inflammatory markers such as IL-1, IL-6, and TNF-α[89,90].

Other important tissues and organs dysfunction/inflammation

Evidence exists that several other tissues and organs in obesity, T2DM, and insulin resistance states are affected by or involved in systemic inflammation. For example, it is well established that macrophage infiltration is associated with islet inflammation and cell abnormalities in T2DM and obesity[91-93]. Furthermore, the western diet and cultural habits may be part of a vicious cycle that promotes oxidative stress and inflammation in the gut and even the brain[94]. Obesity-related inflammation is enhanced by diminished mucosal barriers and intestinal immune homeostasis[95]. These findings could be attributed to effects on the gut microbiome[96]; the importance of diet on organ-specific and systemic inflammation is apparent in diet-induced models of obesity in which even parts of the brain, in particular the hypothalamic arcuate nucleus, have been affected *via* infiltration of macrophages and increased expression of pro-inflammatory markers[16,97-99]. Finally, even non-metabolic or metabolic regulatory organs seem to be affected, as obesity appears to induce ovarian and kidney inflammation, respiratory hyperresponsiveness, and various hematological consequences[100-103].

IMPACT OF CLASSICAL METABOLIC TREATMENTS ON INFLAMMATION AND THE THERAPEUTIC POTENTIAL OF INFLAMMATORY MODIFICATIONS IN METABOLIC DISEASES

Strategies to tackle obesity, diabetes, and related cardiometabolic diseases include a variety of combinations, including lifestyle changes with dietary and exercise options, anti-obesity, anti-diabetic, and anti-hyperlipidemic medications, bariatric or metabolic surgery, and potentially the use of drugs with anti-inflammatory properties[72,104-107].

Current medicinal therapies for T2DM act in a multitude of ways to improve glycemic control, but they can also be beneficial for the treatment of obesity and related cardiometabolic diseases[23,24,72,107]. Many of these medications, for instance, metformin, may possess anti-inflammatory properties that can be exerted indirectly *via* metabolic control of hyperglycemia and hyperlipidemia and weight loss or by directly impacting the immune system and inflammatory responses[72,107]. Weight loss per se and therapeutic interventions that achieve it, including anti-diabetic and anti-hyperlipidemic medication use, resulted in reduced circulating concentrations of IL-6, IL-8, CRP, and MCP-1 and increased adiponectin concentrations[39,40,108-110]. A recent meta-analysis of the effect of intermittent fasting dietary patterns on plasma concentrations of inflammatory biomarkers found a decrease in CRP in individuals with overweight or obesity, but no changes in IL-6 or TNF-α[111]. A meta-analysis of 116 studies[112], however, found that serum concentrations of CRP, IL-6, and TNF- were significantly lower after bariatric surgery.

Targeting inflammation or inflammatory pathways in general has emerged as a viable alternative to traditional metabolic therapeutic options[72,107]. Anti-TNF therapy has produced contentious results in the treatment of T2DM in humans[72,113]. In animal models of IR and T2DM, inhibition combining anti-TNF and IL-1 was shown to be more effective[107,114]. Favorable effects were recorded with IL-1 blockage alone in a human study[115]. Moreover, salsalate, a prodrug of salicylate, diacerein, an anti-arthritis medication, and hydroxychloroquine, usually used for the treatment of autoimmune diseases, appeared to be beneficial; however, long-term safety profiles for these metabolic diseases are still to be elucidated[72]. Finally, the option of directly altering the pro- or anti-inflammatory activation and the balance of the immune cells within the AT arises as a potential therapeutic option[107].

CONCLUSION

In this brief review, we have demonstrated that inflammatory biomarkers reflecting underlying processes and pathway activations are present in obesity and type 2 diabetes mellitus. The impact of obesity and T2DM on inflammatory pathways appears to be linked to disease progression. Achieving a better understanding of the connection and causality between these factors and the disease risk and progression could give us the opportunity to potentially predict, follow up on, and modify their risk. Further studies are warranted to better understand the underlying pathophysiology and the use of predictive biomarkers in everyday clinical practice. It is necessary to conduct *in vivo* physiological studies to investigate the sequence of events underlying pathophysiological events in various metabolic and regulatory tissues, such as adipose tissue. Such studies can help elucidate whether inflammation precedes metabolic derangements or is mainly a result of perturbations caused by increased adiposity. Furthermore, it is essential to conduct randomized clinical trials that precisely target inflammatory pathways in diverse populations to either confirm or refute these findings.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Proportion of thyroid cancer and other cancers in the democratic republic of Congo

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Abstract

BACKGROUND

Cancer diagnosis is increasing around the world and in the Democratic Republic of the Congo (DRC). The proportion of thyroid cancer has increased over the past three decades. There are very few studies on cancer epidemiology, and in particular on thyroid cancer in the DRC.

AIM

To establish the most recent proportion of thyroid cancer in the DRC compared to other cancers.

METHODS

This is a retrospective and descriptive study of 6106 consecutive cancer cases

listed in the pathological registers of 4 Laboratories in the city of Kinshasa. This study included all cancer cases recorded in the registers between 2005 and 2019.

RESULTS

From a sample of 6106 patients, including all cancer types, 68.3% cases were female and 31.7% were male. Breast and cervical cancer were the most common types of cancer in women and, prostate and skin cancer were the most common types in men. Thyroid cancer was sixth in proportion in women and eleventh in men compared to all cancers. Papillary carcinoma was the most common of thyroid cancers. Rare cancers such as anaplastic and medullary thyroid carcinomas had a proportion of 7% and 2%, respectively.

CONCLUSION

Newer diagnostic tools led to a surge in cancer diagnoses in the DRC. Thyroid cancer has more than doubled its proportion over the last several decades in the country.

Key Words: Thyroid cancer; Papillary carcinoma; Cancer; Democratic Republic of the Congo; Africa; Proportion

Core Tip: Cancer diagnosis has been increasing worldwide. This is also true in Africa, particularly in the second biggest African country. However, there are currently no data on cancer in the Democratic Republic of Country (DRC). This study offers the most updated cancer data in general and thyroid cancer in particular in the DRC. Using this current database, more research can be carried out in the country.

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INTRODUCTION

Thyroid pathology is the most common endocrinopathy worldwide[1] and is mostly represented by goiters and nodules[2,3]. Five to ten percent of thyroid nodules are malignant[3-6]. Thyroid cancer only represents 1% of all cancers worldwide[7,8] but has occupied the fifth position among all cancers in France and Canada in women in terms of incidence and twentieth in terms of mortality in 2005[9-11]. The improvement of diagnostic techniques by means of thyroid ultrasound, fine needle aspiration, Computed Tomography scan and detailed histopathological analyses partly explains the increase in incidence of thyroid cancer[12,13]. Despite this increase in incidence, the mortality curve has remained stable over time[10,14].

The Democratic Republic of the Congo (DRC) is a low-income country where there are only 7 pathology laboratories for more than 80 million citizens. Five of these laboratories are located in the capital city of Kinshasa. The typical Congolese meals have been characterized with a low iodine content for decades. Iodine deficiency is a well-known risk factor for thyroid cancer[14,15]. We thus hypothesize that thyroid cancer may be frequent in the DRC but reliable data on cancers in general and thyroid cancer in particular is scarce. The first study addressing thyroid cancer proportion in the DRC was conducted by Mashinda *et al*[16] and it revealed, in women, a thyroid cancer proportion of 0.5% out of all cancers found in the anatomopathological records between 1969 and 2008. Although epidemiologic trend changes are expected to be gradual, the available data now seems dated. The objective of this study is thus to provide more recent thyroid cancer proportion data using the largest series analyzed so far in the DRC.

MATERIALS AND METHODS

This is a retrospective and descriptive study of thyroid cancers and of all types of cancer retrieved from the records of 4 anatomopathological laboratories including that of Kinshasa University Clinics,

National Institute of Biomedical Research, Kinshasa General Hospital (HGRK) and LEBOMA laboratory. All these laboratories are located in the capital city of Kinshasa, a city of nearly 12 million inhabitants. This study included cancers diagnosed in those centers between 2005 and 2019, except for the data obtained from LEBOMA laboratory, which covered from 2015 to 2019. The choice of these centers was governed by the fact that they are the only pathology laboratories in the town of Kinshasa with available data over the period of the study. We calculated the relative proportion of thyroid cancer by dividing the number of thyroid cancers by the number of all types of cancer. It's important to report that calcitonin was not measured preoperatively in patients with thyroid cancer.

The study took into account the following socio-demographic characteristics: Age, gender, year of diagnosis and histopathological diagnosis.

The following types of cancer were taken into account: Breast cancer, cervical cancer, prostate cancer, skin cancer, hematologic cancers, uterine cancer, colon cancer, lung cancer, stomach cancer, bone cancer, thyroid cancer, anorectal cancer, Kaposi sarcoma, soft tissue cancers, eye cancers, ovarian cancer, mouth cancer, vaginal cancer, urinary bladder cancer, laryngeal cancer, nose cancer, peritoneal cancer, liver cancer, renal cancer, vulva cancer, ureteral cancer, nasopharyngeal cancer, intestinal cancer, pancreatic cancer, greater omentum cancer, esophageal cancer, penile cancer, testicular cancer, tonsillar cancer, brain cancer, coecum cancer, vocal cords cancer, ear cancer, parotid glands cancer, duodenal cancer, cancer of the palate, forehead cancer, glottis cancer, trachea cancer, sweat glands cancer, maxillary cancer and splenic cancer.

Data was entered into Excel and transported to Statistical Package for the Social Sciences version 21. Quantitative variables were expressed as mean (+/- SD) or median (+/- interquartile range) for variables that did not have a normal distribution. Qualitative variables were expressed as proportions. Student's *t*-test was used for comparing averages between men and women. χ^2 test or Fisher's exact test was used to compare the difference in proportions between the 2 groups regarding qualitative variables. A P value < 0.05 was of statistical significance.

RESULTS

6106 cancer cases were included in this study. A female predominance was observed with 68.3% of cases *vs* 32.7% for men with a female/male ratio of 4. We noted 106 cases of thyroid cancer, representing 1.7% of the total number of cancer cases. The proportion of all types of cancer according to their location and gender, are grouped together in Table 1.

The most common types of cancer, in decreasing order of proportion were breast cancer, cervical cancer, prostate cancer, skin cancer and lymphoid cancers. Thyroid cancer ranked eleventh in proportion for all cancer types.

The five most common types of cancer in women were breast cancer, cervical cancer, uterine cancer, skin cancer and lymphoid organ (LO) cancer. Thyroid cancer was ranked Sixth with a proportion of 2%.

The five most common types of cancer in men were prostate cancer, skin cancer, LO cancer, lung cancer and colon cancer. For men, thyroid cancer was ranked eleventh (1.1% of all types of cancer).

The male gender was more represented in the age groups ≤ 30 and > 60, while the female gender was more represented in the age groups between 30 and 60 years old. Most cases of cancer in women occur between the ages of 40 and 60 and in men over 50. The number of patients diagnosed with cancer increases with age in both genders.

In the age group up to 40 years old and between 41 and 60 years old, breast cancer was the most common type of cancer. In the age group of over 61, prostate cancer was the most common. Thyroid cancer occupied the sixth position in the age group under 41 years, the tenth in the age group between 41 years and 60 years and the sixteenth in the age group over 60 years old. Figure 1 represents cancer cases' distribution based on age and gender.

The frequencies of cancer cases according to age groups are found in Table 2.

Papillary carcinoma was the most common type of thyroid cancer, representing 67% of all thyroid cancer cases followed by the follicular type in 21% of cases. The anaplastic type occupied the third, lymphoma the fourth and medullary cancer the fifth position.

The frequencies of the different types of thyroid cancer cases in this series are found in Table 3.

DISCUSSION

The main objective of this research was to establish the proportion of thyroid cancer in the Congolese population, secondarily aiming to identify the most frequent types of cancer. Our series included 6106 cases, of which 68.3 % were female and 31.7% were male. This predominance of the female gender was also found in the series of Mashinda who studied the proportion of cancer in the urban setting of Kinshasa[16] and it was also the case for the Lukanu series, which included cases from a rural setting of Congo in Kimpese[17]. These are the first two epidemiological studies analyzing neoplastic diseases carried out in the DRC and which preceded our research.

Table 1 Cancer distribution by affected organs and gender

	All, *n* = 6106	Females, *n* = 4169 (68.3%)	Males, *n* = 1937 (31.7%)
Breast	1631 (26.7)	1560 (37.4)	71 (3.7)
Cervix	1138 (18.6)	1138 (27.3)	-
Prostate	678 (11.1)	-	678 (35)
Skin	356 (5.8)	186 (4.5)	170 (8.8)
Blood and LO	206 (3.4)	95 (2.3)	111 (5.7)
Uterus	196 (3.2)	196 (4.7)	-
Colon	173 (2.8)	92 (2.2)	81 (4.2)
Lungs	161 (2.6)	60 (1.4)	101 (5.2)
Stomach	112 (1.8)	45 (1.1)	67 (3.5)
Bone	110 (1.8)	58 (1.4)	52 (2.7)
Thyroid	106 (1.7)	84 (2)	22 (1.1)
Anus/rectum	97 (1.6)	51 (1.2)	46 (2.4)
KS	87 (1.4)	22 (0.5)	65 (3.4)
Soft tissue	87 (1.4)	52 (1.2)	35 (1.8)
Eye	77 (1.3)	35 (0.8)	42 (2.2)
Ovary	77 (1.3)	77 (1.8)	-
Mouth	76 (1.2)	44 (1.1)	32 (1.7)
Vagina	75 (1.2)	75 (1.8)	-
Urinary bladder	73 (1.2)	32 (0.8)	41 (2.1)
Larynx	65 (1.1)	11 (0.3)	54 (2.8)
Nose	62 (1.0)	28 (0.7)	34 (1.8)
Peritoneum	51 (0.8)	24 (0.6)	27 (1.4)
Liver	49 (0.8)	24 (0.6)	25 (1.3)
Kidneys	41 (0.7)	21 (0.5)	20 (1)
Vulva	41 (0.7)	41 (1)	-
Ureter	33 (0.5)	14 (0.3)	19 (1)
Pharynx/nasopharynx	33 (0.5)	19 (0.4)	14 (0.7)
Intestines	29 (0.5)	16 (0.4)	13 (0.7)
Pancreas	29 (0.5)	13 (0.3)	16 (0.8)
Greater omentum	22 (0.4)	15 (0.4)	7 (0.4)
Esophagus	21 (0.3)	8 (0.2)	13 (0.7)
Penis	20 (0.3)	-	20 (1)
Testicles	20 (0.3)	-	20 (1)
Tonsils	13 (0.2)	5 (0.1)	8 (0.4)
Brain	10 (0.2)	6 (0.1)	4 (0.2)
Coecum	10 (0.2)	4 (0.1)	6 (0.3)
Vocal cords	8 (0.1)	1 (0.0)	7 (0.4)
Ears	7 (0.1)	5 (0.1)	2 (0.1)
Parotid glands	7 (0.1)	2 (0.0)	5 (0.3)
Duodenum	6 (0.1)	2 (0.0)	4 (0.2)
Palate	4 (0.1)	4 (0.1)	0 (0.0)

Forehead	2 (0.0)	1 (0.0)	1 (0.1)
Glottis	2 (0.0)	0 (0.0)	2 (0.1)
Trachea	2 (0.0)	1 (0.0)	1 (0.1)
Sweat glands	1 (0.0)	1 (0.0)	0 (0.0)
Maxillary	1 (0.0)	1 (0.0)	0 (0.0)
Spleen	1 (0.0)	0 (0.0)	1 (0.1)

LO: Lymphoid organ; KS: Kaposi Sarcoma.

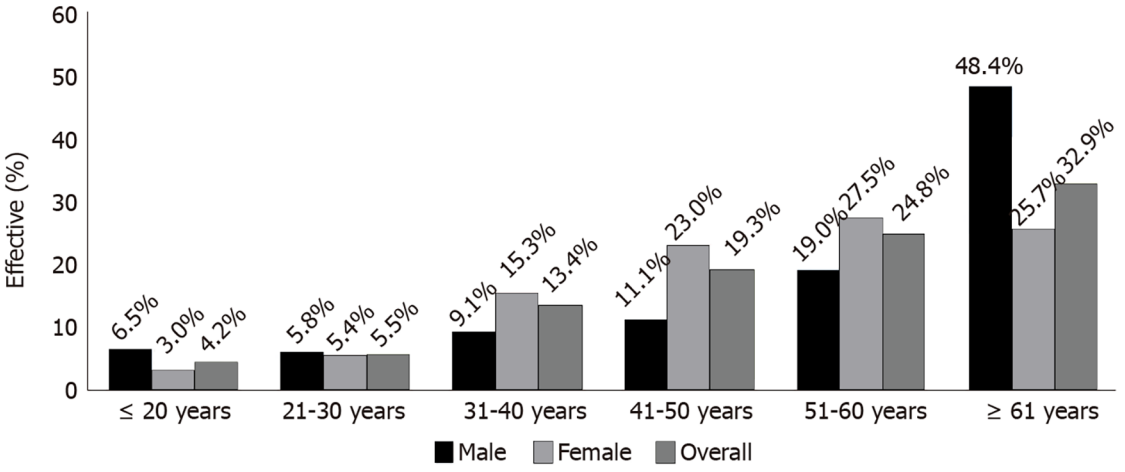

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Figure 1 Cancer distribution based on age and gender.

Female predominance in overall cancer diagnosis was observed in the DRC, a similar trend was also observed in Brazil[18]. The cascade of sexual hormone activity, in particular estrogens and the aromatization of androgens *via* Mitogen-activated protein kinase, causing the decline of immune cells and promoting the proliferation of cancer cells and the inhibition of apoptotic activity can explain the female predominance[19,20]. However, some European series have found a male predominance[21-25].

The male gender was more represented in the age groups up to 30 years and over 60 years; on the other hand, the female gender was more represented in the age groups between 30 and 60 years. Most cancers in women occurred between the ages of 40 and 60 and in men over 50. Overall, 77% of cancer patients were over 40 years of age. Most series around the world, according to which the older the age the greater the probability of developing a neoplastic disease[26,27].

In our series, breast cancer occupies the first position in terms of proportion of cancer in women and cervical cancer occupies the second position. However, in the series published by Mashinda *et al*[16], cervical cancer was the most frequent followed by breast cancer. This difference can be explained by the methodology, the study period, the progress of the national policy on cancer screening in women and by the development of diagnostic means. Mashinda studied the records of two pathology laboratories, while we researched the records of 4 pathology laboratories. Mashinda analyzed results from 1965 to 2008, while we studied data between 2005 and 2019. We must consider that the means of raising awareness have evolved and the educational level of the population has increased over time. All these parameters can explain this difference. The findings of our study are similar to the Lukanu series; breast cancer was the most frequent followed by cervical cancer[17]. Our results also mirrored those found in several African and worldwide series[28-31].

When we consider both sexes, breast cancer was the most frequent type of cancer in our series, this result being similar to those in the literature[29].

Regarding cancer in male patients, prostate cancer was the most frequent in our series. This result was similar to the series of Lukanu[17]. On the other hand, Mashinda found lymphoid organ cancer as the most frequent followed by prostate cancer[16]. Our results are similar to those found in the literature [30]. There has been an improvement in the awareness of the Congolese population regarding prostate cancer over the past two decades.

Thyroid cancer is the most common type of cancer of the endocrine system[32]. Our series found a proportion of 1.7%. In women, the proportion is 2% and it is 1.1% in men, with a female/male ratio of 4. The series described by Mashinda *et al*[16] found a thyroid cancer proportion of 0.5% in women. The

Table 2 Cancer distribution by age group

Cancers	≤ 40 yr, *n* (1409)	%	41-60 yr, *n* (2689)	%	≥ 61 yr, *n* (2008)	%
Prostate	25	1.8	122	4.5	531	26.4
Cervical	156	11.1	611	22.7	371	18.5
Breast	403	28.6	895	33.3	333	16.6
Skin	141	10.0	133	4.9	82	4
Uterus	22	1.6	107	4.0	67	3.3
Lungs	30	2.1	64	2.4	67	3.3
Blood and LO	106	7.5	48	1.8	52	2.6
Colon	37	2.6	92	3.4	44	2.2
Stomach	19	1.3	60	2.2	33	1.6
KS	32	2.3	24	0.9	31	1.5
Soft tissue	27	1.9	30	1.1	30	1.5
Vessels	13	0.9	32	1.2	28	1.4
Bones	51	3.6	33	1.2	26	1.3
Vagina	16	1.1	33	1.2	26	1.3
Larynx	18	1.3	21	0.8	26	1.3
Anus-rectum	27	1.9	45	1.7	25	1.2
Thyroid	37	2.6	45	1.7	24	1.2
Mouth	28	2.0	24	0.9	24	1.2
Liver	12	0.9	19	0.7	18	0.9
Ureter	3	0.2	12	0.4	18	0.9
Vulva	4	0.3	20	0.7	17	0.8
Ovary	25	1.8	36	1.3	16	0.8
Peritoneum	12	0.9	23	0.9	16	0.8
Nose	26	1.8	23	0.9	13	0.6
Eye	35	2.5	30	1.1	12	0.6
Esophagus	5	0.4	6	0.2	10	0.5
Testicles	8	0.6	2	0.1	10	0.5
Pharynx	14	1.0	8	0.3	9	0.4
Pancreas	5	0.4	16	0.6	8	0.4
Intestines	7	0.5	15	0.6	7	0.3
Kidney	23	1.6	11	0.4	7	0.3
Vocal cords	1	0.1	2	0.1	5	0.2
Greater omentum	7	0.5	11	0.4	4	0.2
Penis	5	0.4	12	0.4	3	0.1
Duodenum	1	0.1	2	0.1	3	0.1
Tonsils	4	0.3	7	0.3	2	0.1
Coecum	5	0.4	3	0.1	2	0.1
Ears	4	0.3	1	0.0	2	0.1
Palate	1	0.1	1	0.0	2	0.1
Parotid glands	3	0.2	3	0.1	1	0.0
Trachea	1	0.1	0	0.0	1	0.0

Maxillary	0	0.0	0	0.0	1	0.0
Spleen	0	0.0	0	0.0	1	0.0
Brain	6	0.4	4	0.1	0	0.0
Glottis	0	0.0	2	0.1	0	0.0
Nasopharynx	1	0.1	1	0.0	0	0.0
Forehead	2	0.1	0	0.0	0	0.0
Sweat glands	1	0.1	0	0.0	0	0.0

LO: Lymphoid organ; KS: Kaposi Sarcoma.

Table 3 Frequency and distribution of the cases of thyroid cancer according to histological type

Variables	Number of cases	Percentage (%)
Histology		
Papillary carcinoma	71	67.0
Follicular carcinoma	23	21.7
Anaplastic carcinoma	8	7.5
Lymphoma	3	2.8
Medullary carcinoma	1	0.9

proportion of thyroid cancer in women in our series compared to that of Mashinda's is multiplied by 4, a female predominance that is confirmed in the literature[33,34]. Thyroid cancer occupied the sixth position of all listed cancers among women in our series, whereas it is the 5th most frequent cancer in women worldwide[35].

The increase in thyroid cancer proportion has also been observed in several studies around the world over the past three decades[10]. The mechanisms underlying this increase have not yet been elucidated. However, nutritional, hormonal, anthropometric, environmental, and other factors are suspected. Many authors also consider that excessive iodine intake, and the development and accessibility of diagnostic tools participate to the increase in diagnosis[36,37].

Our study found that nearly 7% of thyroid cancers were anaplastic and 1% were medullary, while these cancers are rare in the literature[38]. This can be considered as a particularity of the DRC regarding thyroid cancer, especially since calcitonin is not generally measured in the assessment of thyroid nodules or preceding thyroidectomy.

It is known that 90% of thyroid cancers are differentiated and have good prognosis and that only 5% to 10% are undifferentiated and have therefore a bad prognosis[39,40]. This high proportion of undifferentiated cancers in our series constitutes a particularity of the Congo. This can be explained by the fact that, undifferentiated thyroid cancers, given their aggressive behavior, are more likely to warrant a surgical evaluation. Thyroid cancers in our study originated from surgical pathology reports. Nevertheless, this particularity requires more in-depth studies to better understand the causes and mechanisms.

Since iodine deficiency in the soil is considered a risk factor for anaplastic cancer[41], the question to be raised is whether iodine deficiency could be responsible for this higher proportion of undifferentiated cancers. knowing that iodine saturation in the Congo was only obtained in 1993[42]. Another potential mechanism is that initially differentiated cancers have lost differentiation over time[43] due to late diagnosis.

This work has the limitations of retrospective studies. In addition, it is biased due to the fact that we only took into consideration the patients who had carried out the anatomopathology while those who had not carried out one, were not included in this study, this may have influenced a high proportion of cancers and certain histological types. Finally, limitations in diagnostic facilities in data reporting in a resource-poor healthcare facility are also potentially limiting.

Despite these limitations, this work gives a scoping vision of cancer in the DRC and in particular of thyroid cancer. It has established the frequencies of different forms of cancer in a country where cancer data are rare.

CONCLUSION

Cancer diagnosis is on the rise in the DRC and the proportion of thyroid cancer as compared to total number of cancers has doubled over the period from 2005 to 2019. A marked female predominance was observed. Papillary thyroid cancer is the most frequent type of thyroid cancer followed by follicular carcinoma. There is a high proportion of undifferentiated thyroid cancers such as anaplastic carcinomas, long recognized as rare carcinomas. Breast cancer is the most common of all types of cancer, followed by cervical cancer. Prostate cancer is the most common type of cancer in men. Thyroid cancer ranked sixth most common cancer in women and eleventh most common in men. This study establishes the most recent and updated proportion of thyroid cancer in the second-largest African country.

ARTICLE HIGHLIGHTS

Research background

Cancer diagnosis has been increasing worldwide and in Africa as well, particularly in the Democratic Republic of Congo (DRC). However, there are currently no studies addressing the proportion of different cancers in the DRC, and in particular thyroid cancer.

Research motivation

The main motivation of this study was to identify the proportions of different cancers in the DRC and in particular thyroid cancer.

Research objectives

The purpose of this study was to analyze different epidemiologic characteristics of thyroid cancers in the second-largest African country while establishing the proportions of all cancers in the country.

Research methods

This is a retrospective and descriptive study of 6106 consecutive cancer cases listed in the pathological registers of 4 Laboratories in the city of Kinshasa. This study included all cancer cases recorded in the registers between 2005 and 2019.

Research results

In our series two third of cancer patients were females. Breast is the most common cancer in females while prostate cancer is the most common among their male counterparts. Thyroid cancer was ranked sixth in occurrence and a higher proportion of anaplastic thyroid cancer was encountered.

Research conclusions

Female patients seem to be more affected by cancer than their male counterparts. Rare anaplastic thyroid cancers which are often associated with a dismal prognosis, although rare in the literature, are found in higher proportion in the DRC. A surge of all cancers was also observed owing to the advances in diagnostic tools used.

Research perspectives

Oncology and cancer research in the DRC remains an unexplored area. There is a serious paucity of data of any cancer in the country. This study offers the most updated data on cancer in the second-largest African country. This study paves the way for future prospective studies in the country, helping to identify groups at higher risk and shaping the national guidelines.

FOOTNOTES

Author contributions: Bukasa-Kakamba J and Bangolo AI searched the literature, wrote, and revised the manuscript; Mbunga B, Iyese F, Nkodila A, Atoot A, Anand G, Lee SH, Chaudhary M, Fernandes PQ, Mannam HPSS, Polavarapu A, Merajunnissa M, Azhar A, Alichetty MN, Singh G, Arana Jr GV, Sekhon I, Singh M, Rodriguez-Castro JD revised and edited the manuscript; Atoot A, Bayauli P, Weissman S and M'Buyamba JR revised and approved the final version and are the article's guarantors; all authors certify that they contributed sufficiently to the intellectual content and data analysis. Each author has reviewed the final version of the manuscript and approves it for publication.

Institutional review board statement: This study protocol was reviewed and approved by the national health ethics committee of the DRC, number 197/CNES/BN/PMMF/2020.

Informed consent statement: The dataset used in this study was de-identified, thus the need for patients' informed consent was waived by the Congolese Health Ethics Committee.

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Role of children in the Bulgarian COVID-19 epidemic: A mathematical model study

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Abstract

BACKGROUND

The coronavirus disease 2019 (COVID-19) pandemic affects all aspects of our lives, including children. With the advancement of the pandemic, children under five years old are at increased risk of hospitalization relative to other age groups. This makes it paramount that we develop tools to address the two critical aspects of preserving children's health – new treatment protocols and new predictive models. For those purposes, we need to understand better the effects of COVID-19 on children, and we need to be able to predict the number of affected children as a proportion of the number of infected children. This is why our research focuses on clinical and epidemiological pictures of children with heart damage post-COVID, as a part of the general picture of post-COVID among this age group.

AIM

To demonstrate the role of children in the COVID-19 spread in Bulgaria and to test the hypothesis that there are no secondary transmissions in schools and from children to adults.

METHODS

Our modeling and data show with high probability that in Bulgaria, with our current measures, vaccination strategy and contact structure, the pandemic is driven by the children and their contacts in school.

RESULTS

This makes it paramount that we develop tools to address the two critical aspects of preserving children's health – new treatment protocols and new predictive models. For those purposes, we need to understand better the effects of COVID-19 on children, and we need to be able to predict the number of affected children as a proportion of the number of infected children. This is why our research focuses on clinical and epidemiological pictures of children with heart damage post-COVID, as a part of the general picture of post-Covid among this age group.

CONCLUSION

Our modeling rejects that hypothesis, and the epidemiological data supports that. We used epidemiological data to support the validity of our modeling. The first summer wave in 2020 from the listed here school proms endorse the idea of transmissions from students to teachers.

Key Words: COVID-19; Pandemic; Children; Cardiac involvement; Multisystem inflammation in children; ARIMA; Time-series modeling; Regression model

Core Tip: The lack of vaccination strategy accelerates the spread of coronavirus disease 2019 among children and accelerate the spread among people below 50 years. Based on the latter hypothesis and the other three: (1) Disease spreads from children to adults – either directly to parents and grandparents or *via* network spread to different age groups; (2) the spread among children accelerates with the increasing R0 of different dominating viral variants; and (3) vaccinations among adults accelerate the spread among the less vaccinated group of children, we outlined the reasons for this age-restricted acceleration of the spread after the delta wave.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic impacts all aspects of our lives, including the increased burden on the healthcare system, altered work, education, social life, etc. The pandemic also affects children. They are at increased risk for type 1 diabetes post-COVID, even more, pronounced in children than the adult patients[1]. Additionally, they have persistent lung damage[2]. With the advancement of the pandemic, children under five years old are at increased risk of hospitalization relative to other age groups that have immunity from vaccination and/or infection[3]. This makes it paramount that we develop tools to address the two critical aspects of preserving children's health – new treatment protocols and new predictive abilities. For those purposes, we need to understand better the effects of COVID on children, and we need to be able to predict the number of affected children as a proportion of the number of infected children. This is why our research focuses on clinical and epidemiological pictures of children with heart damage post-COVID, as a part of the general picture of post-COVID among this age group.

COVID-19 and children

Although severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was previously considered clinically milder in children compared to adults, the severe consequences of COVID-19, known as multisystem inflammation in children (MIS-C), has become increasingly significant with the accumulation of practical experience worldwide. As we recently published, MIS-C is associated mainly with late post-viral immunological responses leading to multiorgan impairment and failure[4,5].

Except for liver involvement, cardiac damage is also typical in children after asymptomatic and symptomatic COVID-19, as myocardial, pericardial, and/or coronary manifestations. Recently, we presented the results from the retrospective analysis of pediatric patients with cardiovascular system

involvement in 31.4% of confirmed COVID-19 cases admitted to the hospital[6,7]. The most common clinical features were acute myocarditis with severe contractile dysfunction, acute heart failure, and left main coronary artery dilation; systolic dysfunction, dilation and regional hypokinesia of the left ventricle; acute vasculitis with transient dilation of the coronary arteries; pericarditis as part of a pronounced polyserositis syndrome[6]. It has been demonstrated that children with pre-existing comorbidities (cardiovascular diseases, cancer, renal failure) are at risk for severe COVID-19[8,9].

Fortunately, the course of the disease after the complex treatment (immunomodulatory therapy with intravenous immunoglobulins and corticosteroids, inotropic drugs and anticoagulants) was favorable in all patients. Furthermore, although the COVID-19 therapy is non-specific, to some extent in some cases it may lead to further complications (*i.e.*, liver injury, *etc.*)[10].

In the initial phase of the Covid-19 pandemic, some studies claimed that children rarely get COVID-19 and do not spread as much as adults *via* schools[11]. The airborne nature of the disease was also disputed[12], commenting also the short-range aerosol transmission and while masking will not decrease exposure to the virus to zero, it offers incremental benefits that, when combined with other strategies, will lower the risk of infection even more.

For this reason, very few preventive measures in schools in countries such as Bulgaria were implemented in 2020[13]. Transmission from children to adults was deemed secondary to the transmission from adults to children. After the first big wave in September to December 2020, masks were mandated in grades 5 and above but not enforced, with arguably low adherence, less than the general adherence[14] due to mask removal in breaks between classes.

We use mathematical modeling (time series analysis) to test the following hypotheses in the case of Bulgaria and to show the price of the lack of policy in schools: (1) The possibility of emerging variants to accelerate their spread among people below 50 years, thus compensating for the effect of acquired partial herd immunity; (2) disease spreads from children to adults – either directly to parents and grandparents or *via* network spread to different age groups; (3) the spread among children accelerates with the increasing R0 of different dominating viral variants; and (4) vaccinations among adults accelerate the spread among the less vaccinated group of children. Our modeling can be used for both explanations of dependency and short-term prediction of a future number of cases. It is also potentially applicable to small datasets, such as the MIS-C cases at Pirogov Hospital, as we show in this paper.

Epidemiological data for spread among age groups in Bulgarian children

With starting the pandemic in Bulgaria, children were mostly speared from infection. On the one hand, it was thought that COVID-19 took a mild course in children. On the other hand, the testing was at the lowest rate among children. Up to now, the total number of infected children (0-18 years) is 103743[15].

We aimed to demonstrate the role of children in the COVID-19 spread in Bulgaria and to test the hypothesis that there are no secondary transmissions in schools and from children to adults. Our modeling rejects that hypothesis, and the epidemiological data supports that. We use epidemiological data to support the validity of our modeling. The first summer wave in 2020 from the listed here school proms endorses the idea of transmissions from students to teachers.

MATERIALS AND METHODS

Statistical modeling - methods and hypotheses

In our previous research[16], we used ARIMA models to predict deaths weekly from new cases among different age groups. The approach is suitable for time series analysis with time-varying effective reproductive number Rt. Moreover, it allows us to regress against other predictors to test some hypotheses which set of factors influences the spread of the disease. For children, we have several hypotheses.

The most important one is the significant influence of school closure on the number of new cases. The second in order of importance is that the disease spreads from children to adults – either directly to parents and grandparents or *via* network spread to different age groups. The third hypothesis is that the spread among children accelerates with the increasing R0 of different dominating viral variants. Since the R0, or basic reproduction number[17], is the number of secondary cases of a disease that result from the emergence of a single index case in a population of uniformly dispersed susceptible people, therefore, if the R0 is less than one, it indicates that the epidemic is under control; if it is larger than one, it indicates that the disease is spreading.

The fourth hypothesis is that vaccinations among adults accelerate the spread among the less vaccinated group of children. By acceleration, we mean the increased proportion of susceptible children with the vaccinations of the adults that redirects the spread among them. The fifth hypothesis, which is not self-evident, is that the vaccinations, when done uniformly across age groups above 0-19 age group, have positive feedback on the spread among the age groups over 60. Our basis for this last hypothesis is the difference in mobility and contacts by age group. Preferential vaccination of the age groups with higher contribution to the spread should decrease it to a larger degree than uniformly distributed vaccination if the vaccines slow the spread – if they convey some protection from infection.

Although our modeling is statistical in nature, there are autoregressive components in ARIMA models that should count for the effects of the accumulating number of survivors with their protection against reinfection. Although this number is subject to considerable uncertainty and is limited by our knowledge for the reinfections with SARS-CoV-2, seasonal coronaviruses induce short-lived immunity and we might expect a similar pattern here[18].

The accumulated protective immunity in the form of total cases could be added as a factor, but it has a high correlation with emerging variants. The emergence of new variants is the consequence of that increase in immunity and the co-evolution of humans and the pathogen, seeking equilibrium. This is one reason to expect the role of variants to be harder to detect in our models and to add only them as a factor. They also represent the net effect of acquired immunity. Any detectable influence will be the result of the net balance between increased virulence and immune escape of the viral variants vs the acquired immunity on a population level.

Data and preparation

We use the official weekly date from the Unified National Portal in Bulgaria[19]. New cases are split into age groups - 0-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80-89, and 90+ years old. For vaccinations, we use data from [Our World In Data, Coronavirus (COVID-19) Vaccinations, https://ourworldindata.org/covid-vaccinations?country=BGR]. For the dominance of other virus variants, we use data from National Centre for Infectious and Parasitic diseases[20]. Finally, we use the Oxford stringency index data for school closures[21]. The data is categorical – 0-1-2-3 for different levels of school closures.

The data for new cases in different age groups is on a daily basis and is cumulative. We differentiate it and aggregate it weekly with the help of the open-source platform Octave in the interval between June 7, 2020 (week 23) and November 8, 2021 (week 44). We have a set of 74 wkly observations. As can be seen from the data (Figure 1), there is a very clear lag between new cases for 0-19 years, and other age groups – a consecutive movement from lower to higher age groups can be seen before any modeling being done.

The sample crosscorrelation function between new cases for 0-19 year and the MIS-C cases show similar behavior with even larger lags up to 40 d (Figure 2 and 3) which shows the potential usefulness of a model, that describes the pandemic spreads among age groups. Children with MIS-C are lagging further behind the cases among adults.

From the updated Principal Component Analysis (Figure 4), we can see that the 0-19 age groups stand apart from others, and other clear groups are identified – 20-49 years and 60-89 years. For our purposes, here we aggregate 20-49 years and 60-89. We leave 50-59 and 90+ due to a weaker correlation. Our hypothesis for 90+ is that these people live isolated from relatives, often in nursery homes or villages with weak traffic outside of summers, where seasonality decreases the spread. For the 50-59 age group, this is the age group in which children have grown up and are no longer in school. This is one possible explanation for the weaker connection to the 0-19 age group.

We used vaccines and variants data to create factor variables. For vaccines, 0%-9.99% of fully vaccinated people are with value 0%, 10%-19.99% are with 1%, and 20%-29.99% are with 2. For variants, the wild type from Wuhan is with 0; alpha is with 1, and Delta is with 2. Therefore, we do not consider it, for now, Delta+ separately, but when it gets dominant, we can add another value, 3, and we will update our model. As we showed in our previous research on which we step here, there are serious correlations between new cases in different age groups, which is the reason why we aggregate cases by combining age groups.

Factors contributing to the spread of COVID-19 among children

First, we investigated the spread among children. To test it, we used regression with ARIMA errors. Like in our previous research, we use R, with packages "forecast" and "urca". We examine for stationarity the time series with two different tests - Kwiatkowski-Phillips-Schmidt-Shin (KPSS) tests[22] and the Augmented Dickey-Fuller test (ADF)[23].

In the case of weekly data for 0-19 years old, both tests gave an order of differentiation 1. Therefore, we used the ARIMA model with fixed order of differentiation 1 and the auto for our purposes.arima() function[24] to select the best possible model. Our regressors are the variants factor and the vaccines factor. In addition, the same function estimates the dynamic regressions by adding the predictors thanks to the argument "xreg." After some additional lag selection of the chosen regressors, based on RMSE, MAPE, MAE, AIC, practical considerations and the standard errors of the coefficients, we obtained an optimal model - Model I (Tables 1 and 2, Figures 5 and 6) based on minimal data errors covering the period from June 7, 2020, to November 2, 2021.

Temporal spread across age groups

One of our significant hypotheses is the spread across age groups – from children to parents and grandparents in households and by secondary contacts. There are clear lags, shown in Figure 1, for which we model here. We separate the other age groups into two main groups – the group of parents 20-49 and the group of grandparents with contacts to children 60-89. The age group 50-59 is largely devoid of direct contact with children or grandchildren, and the same is for 90+. We used second-order

Table 1 Correlations between the variables for Model I – factors contributing to the spread among children

Variables	Variants	Vaccines	Schools L1	Schools L2
Variants	1	0.7877448	-0.4553950	-0.4553950
Vaccines	0.7877448	1	-0.4089070	-0.4502049
Schools L1	0.4553950	-0.4089070	1	-0.4089070
Schools L2	0.4553950	0.4502049	0.8506835	1

Table 2 Regression models with ARIMA (0,1,1) errors - Model I – factors contributing to spread among children

Model summary		
Coefficient	**Estimate**	**Standard error**
MA1	0.4995	0.1272
Variants[a]	71.0883	98.4872
Vaccines L2	**191.2172**	114.5689
Schools L1	**-103.5798**	57.1372
Schools L2	-37.4081	56.7021
R^2	**0.926**	
RMSE	**154.84**	
Bias	**8.79**	
MAPE	**43.35**	

[a]This variable is not so well estimated because the newest variant has not enough data.

differencing on our model to account for quadratic growth[25] and decrease the correlation between predictors to acceptable levels. Using the similar criteria as in Model I, we receive optimal model - Model II (Tables 3 and 4, Figures 7 and 8)

For our third model – Model III, we use the same regressors, but this time against the new weekly cases for the age groups 20-49 (Table 5, Figures 9 and 10).

Epidemiology of MIS-C in Pirogov: Characteristics of MIS-C dynamics and modeling approach

On the individual level, the onset of symptoms from MIS-C is, on average, 5 wk (4 to 6 wk) after the acute phase of the disease[26]. On the regional level, however, we see very clearly much more significant delays from cross-correlation analysis between new cases of 0-19 years old and new cases of MIS-C on a weekly basis (Figures 2 and 3). The delays are 41-48 wk between new cases and MIS-C cases. One possible explanation for this is that MIS-C is a very low probability event, which means that many covid-19 cases are necessary for one mis-c case to happen, and a lot of time must pass to accumulate these cases. Another possible explanation is that the analysis included data from a single center in the capital of Bulgaria, which cannot be fully representative considering that the Department is intensive and urgent but has no cardiology profile.

On the other hand, accumulating data for more than one year can make it possible to search, including the "seasonality" hypothesis. However, when December came, naturally, there was a peak again. Unfortunately, however, we did not find a description of this phenomenon in the literature.

"In this cohort study of 248 persons with MIS-C, MIS-C incidence was 5.1 persons per 1000000 person-months and 316 persons per 1000000 SARS-CoV-2 infections in persons younger than 21 years. Incidence was higher among Black, Hispanic or Latino, and Asian or Pacific Islander persons compared with White persons and in younger persons compared with older persons."[27]. Another more straightforward but less likely explanation is due to the dynamics of repeated waves which are highly correlated with each other (very similar in nature). It is less likely due to the significantly higher correlations between cases and MIS-C for the more considerable lags of 41-48 wk. Whatever the cause, a simple predictive model benefits from the lags with higher correlations.

The epidemiological data includes 4 additional MIS-C cases, a total of 39, which arrived in weeks 77th and 78th since June 6, 2020 (the initial date for new cases by age groups time series), which is November 29, 2021 - December 5, 2021. The relative growth does not display these leading delays (lags) of over 40 wk, which shows why it was necessary to compute the cross-correlation (Figure 2) function in the first place. The ADF shows one root at unity – the process of MIS-C arrival is non-stationary, but its first

Table 3 Correlations between the variables for Model II

Variables	Variants	Vaccines	0-19 L1	0-19 L2	0-19 L3
Variants	1	0.00	-0.08	0	0.00
Vaccines	0	1	-0.19	-0.13	0.34
0-19 L1	-0.08	-0.19	1	-0.40	-0.04
0-19 L2	0	-0.13	-0.40	1	-0.28
0-19 L3	0.00	0.34	-0.04	-0.28	1

Table 4 Regression models with ARIMA (0,2,1) errors - Model II

Model summary		
Coefficient	**Estimate**	**Standard error**
MA1	-0.4661	0.1438
Variants[a]	147.7111	385.2214
Vaccines	**548.6656**	412.7460
0-19 L1	1.2614	0.5521
0-19 L2	**1.4164**	0.5521
0-19 L3	1.1012	0.6543
R^2	**0.962**	
RMSE	**620.16**	
Bias	**-16.44**	
MAPE	**23.38**	

[a]This variable is not so well estimated because the newest variant has not enough data.

Table 5 Regression models with ARIMA (0,2,1) errors – Model III

Model summary		
Coefficient	**Estimate**	**Standard error**
MA1	-0.9502	0.1272
Variants[a]	724.0295	641.8759
Vaccines	**1095.1284**	691.3952
0-19 L1	**5.7357**	0.0519
0-19 L3	1.9966	0.8769
R^2	**0.953**	
RMSE	**897.5266**	
Bias	**-72.74389**	
MAPE	**31.96102**	

[a]This variable is not so well estimated because the newest variant has not enough data.

finite difference is a stationary process. Therefore, at least one differencing is needed for Arima modeling.

Probabilistic modeling of MIS-C dynamics

We can consider the arrival times of children with MIS-C as independent in time. This is possible due to the perceived random nature of the occurrence of MIS-C due to independent factors and the lack of

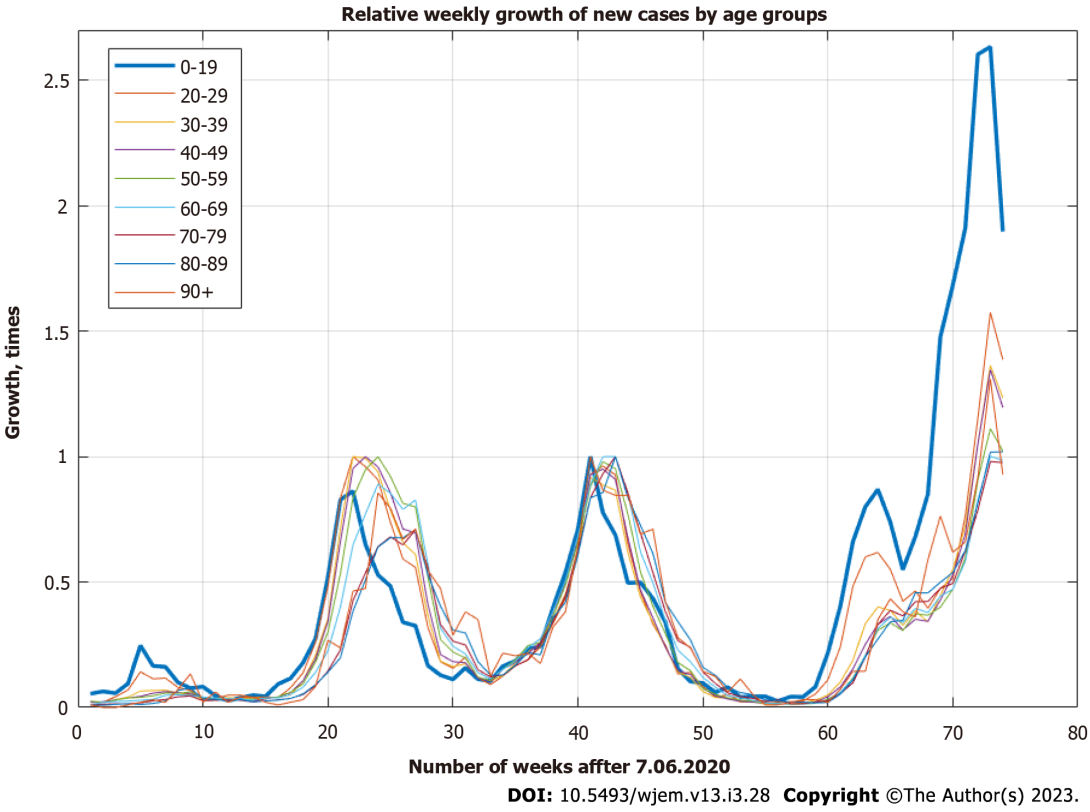

Figure 1 Relative growth of cases by age groups. An apparent lag between 0-19 and others can be seen.

clear predictors of which child will have MIS-C in the post-acute phase. Therefore, the most suitable distribution for independent arrivals is the Poisson distribution.

We have to note that this modeling is most appropriate when the mean value is constant, which is not so during the pandemic, in which the process of MIS-C arrivals is non-stationary. In a post hoc analysis, this Poisson distribution may change its lambda. However, it can still be used to forecast by taking the λ as a parameter in time λ (t) and forecasting its change. Then at every point in time, this distribution can be recalculated, and we can have the expected value and some confidence interval for which the number of arrivals at a given time period will be there with predefined probability (for example, 95% confidence interval).

Time series analysis

To obtain a model that explains the variation relatively well for this small set of data (39 cases), we grouped both new cases for 0-19 on a national level and MIS-C in one center –the Pediatric department and Pediatric ICU in the main Emergency Hospital in the capital city on a bi-weekly basis. This allowed us to have a good evaluation of the NC L21 parameter (Table 6), which shows the new cases 21 bi-weekly periods ago.

In general, the MIS-C cases in Pirogov are 1% of the cases among 0-19, which gives roughly 2%-3% in the respective age group for that disease, which is a subset of 0-19. This is incredibly high compared to the relatively low frequency of MIS-C, as we quoted already. Nevertheless, this can be used to obtain a very rough estimation for the number of children missed by testing if we combine this set with the data from other hospitals in Bulgaria.

RESULTS

Model I - factors contributing to the spread of COVID-19 among children

The optimal model is Arima (0,1,1) (Table 2, Figures 5 and 6) and uses school closures with lags of 1 and 2 wk, variants with lag 0 wk and vaccines with a lag of 2 wk. We ensure that the correlation between the four predictors (regressors) is acceptable, as seen in Table 1. The model summary is given in Table II. The residuals are shown in Figure 5, and the model fit – is in Figure 6. A rule of thumb for a good fit is to have an absolute value of a coefficient twice as significant as its standard error.

As Table 2 shows, school closures and vaccines have significant impacts on the spread among children – 0-19 years old age group. Michos *et al*[28] reported the hospitalization rates of children due to

Table 6 Regression models with ARIMA (0,1,0) errors

Model summary		
Coefficient	**Estimate**	**Standard error**
NC L21	**0.0016**	0.0008
NC L22	-0.0011	0.0007
NC L23	1e-04	7e-04
R^2	**N/A**	
RMSE	**0.8425338**	
Bias	**N/A**	
MASE	**0.4601526**	

N/A: Not applicable.

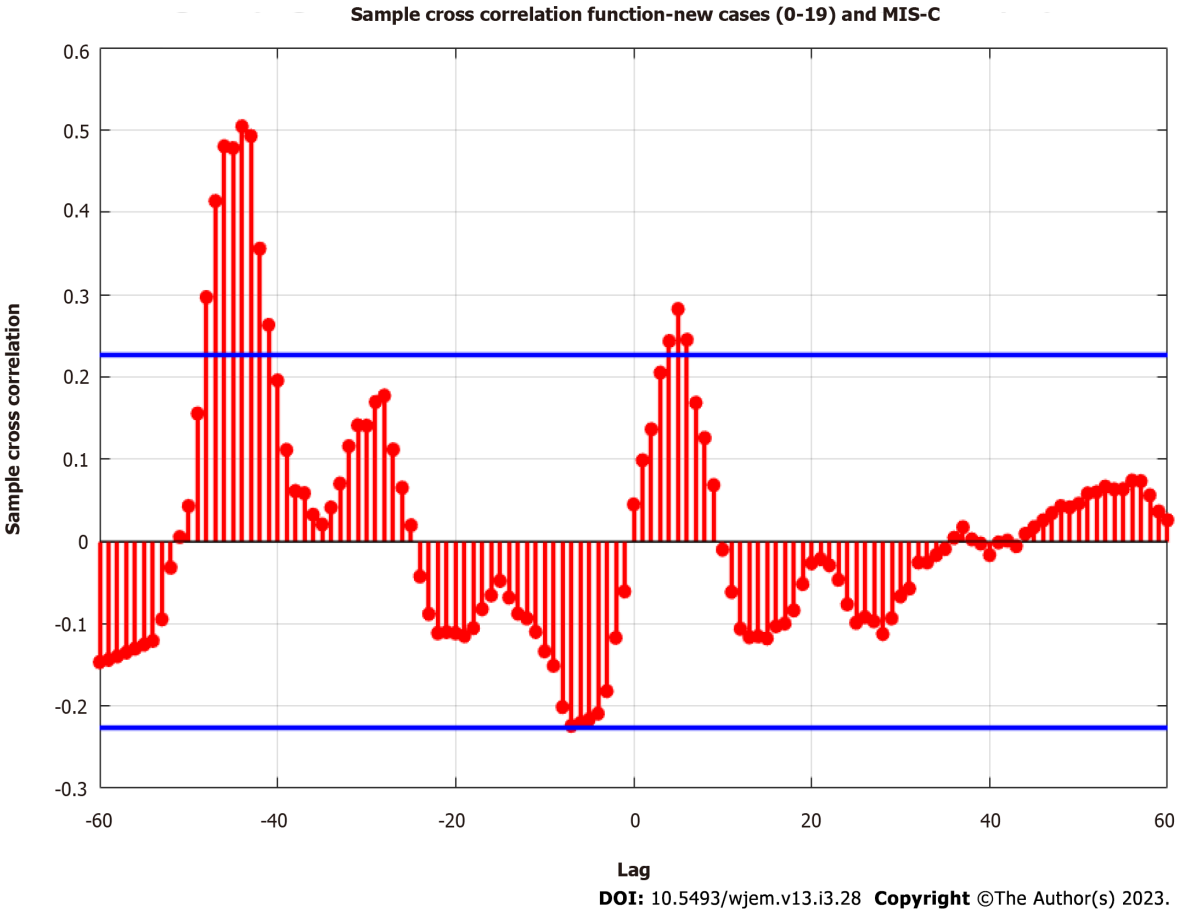

Figure 2 Sample cross-correlation function between new weekly cases of coronavirus disease 2019 and multisystem inflammation in children cases. MIS-C: Multisystem inflammation in children.

COVID-19 in Greece and the United States[29,30].

In terms of age groups, among > 1.2 million children below 18 years of age with SARS-CoV-2 infection in the USA between March and December 2020, children were distributed as follows: Preschool (age 0 through 4 years)–7.4%; elementary school (age 5 through 10 years)–10.9%; middle school (age 11 through 13 years)–7.9%; high school (age 14 through 17 years)–16.3%. However, in Bulgaria, no such information is available.

Closures decrease significantly the numbers – closure of level 3 falls on average with 310 cases per week or a minimum of 30% reduction of the number of cases. It is a well-estimated coefficient with a standard error of half its value. Close to that proper precision of estimation is the Vaccines factor, which gives 192 cases weekly per 10% of fully vaccinated adults or an overall close to 400 cases per week

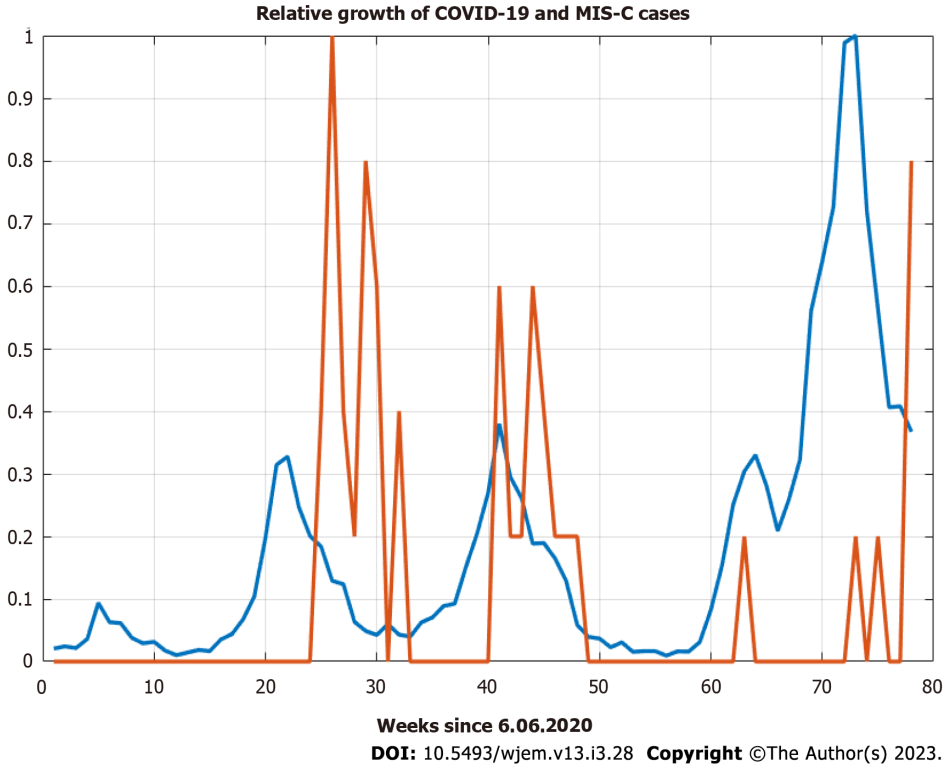

Figure 3 Relative growth of national-level coronavirus disease 2019 cases and multisystem inflammation in children cases in Pirogov Hospital. COVID-19: Coronavirus disease 2019; MIS-C: Multisystem inflammation in children.

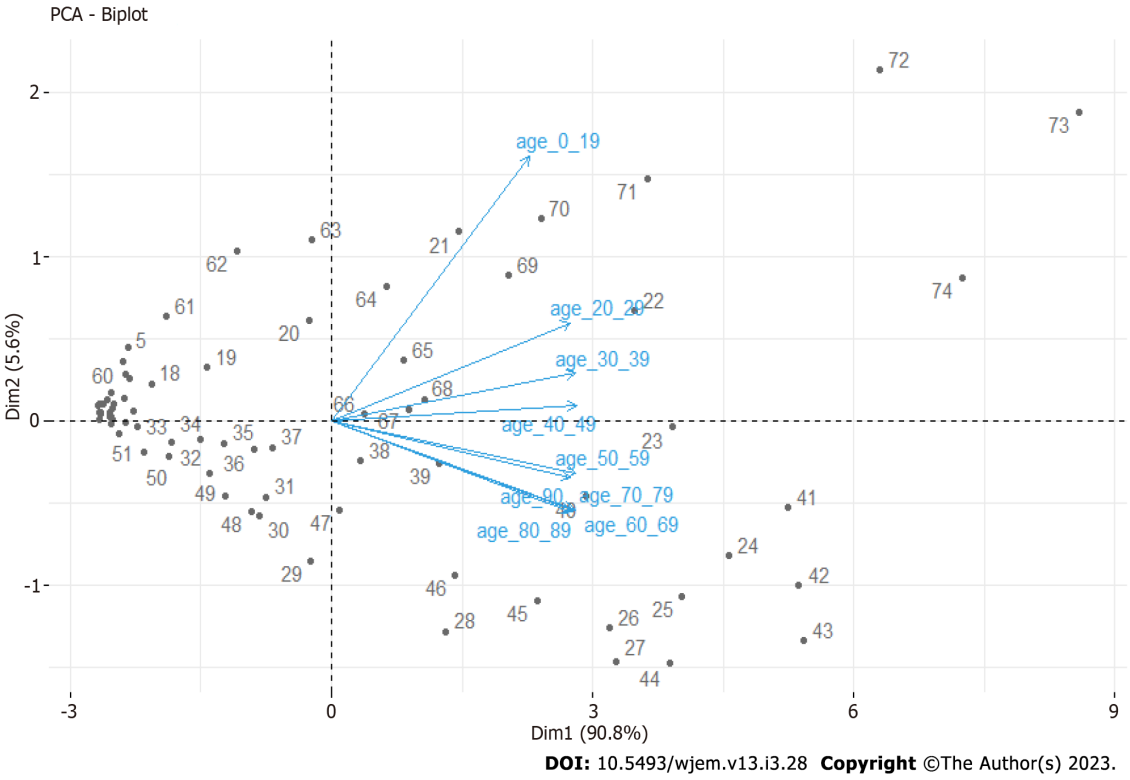

Figure 4 Principal component analysis Biplot for age groups on a weekly basis. PCA: Principal component analysis.

increase. We see here that this is a probable conclusion – vaccinations of adults accelerate the spread among mostly unvaccinated children.

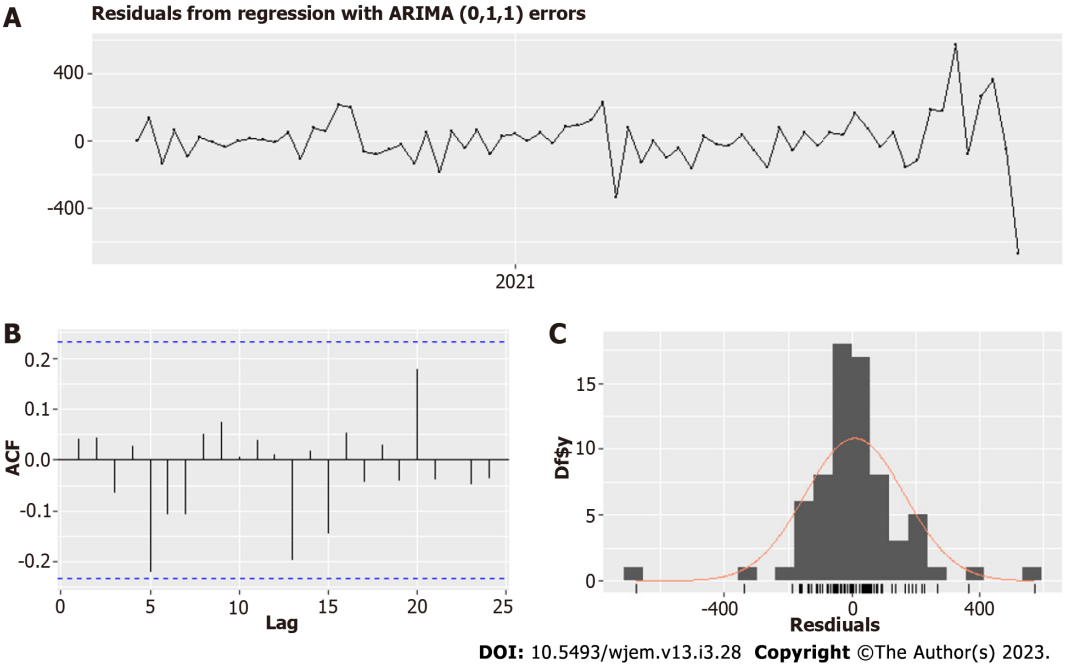

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Figure 5 Residuals of Model I – Factors contributing to the spread among children. A: Residuals of the model; B: Autocorrelation function of the residuals; C: Distribution of the residuals.

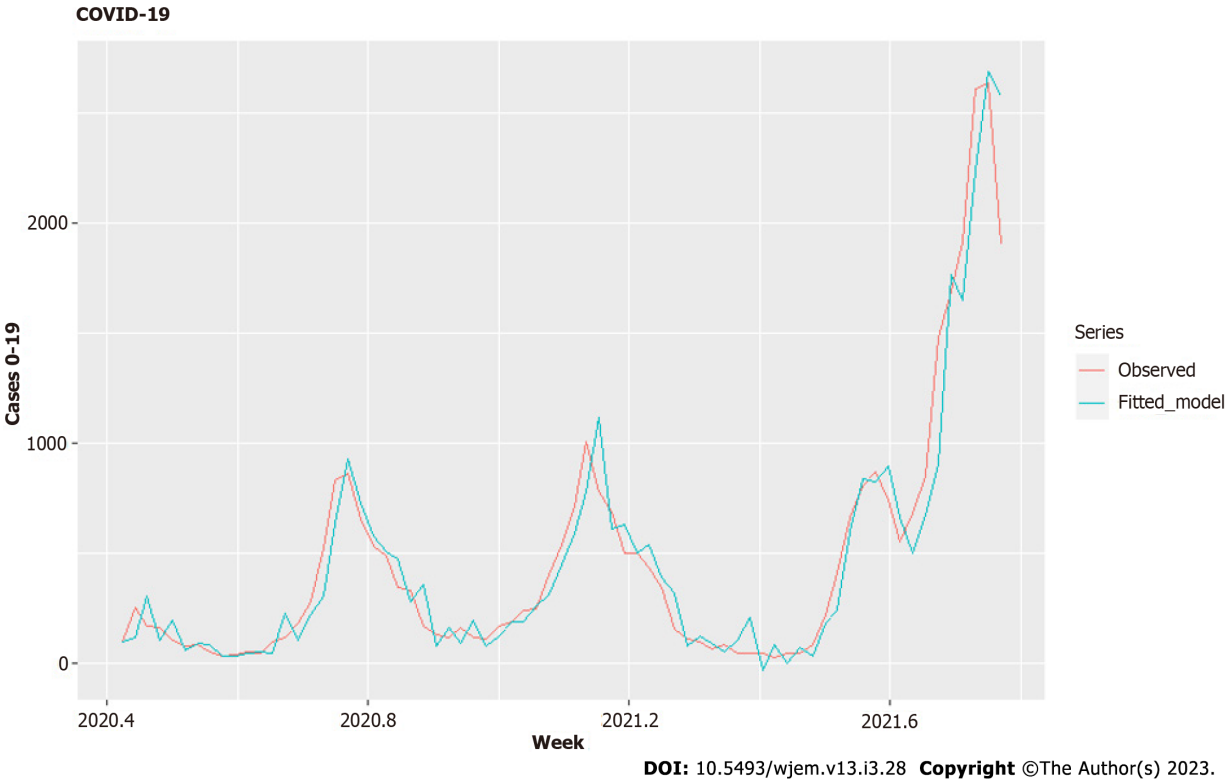

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Figure 6 Fitted Model I – Factors contributing to the spread among children. COVID-19: Coronavirus disease 2019.

Despite all issues with data related to the testing of children in Bulgaria, we managed to explain 93% of the variation with our model. With this currently limited data, the role of variants remains unverified due to a relatively high correlation with vaccinations – they have similar dynamics. There is also an accumulation of immunity through prior infection that competes with the variants' tendency to increase rates among children. We may update or change the model accordingly after the Delta and Omicron wave to include more values to the variants factor and to check whether variants accelerate the spread or distinguish it from the vaccination pressure.

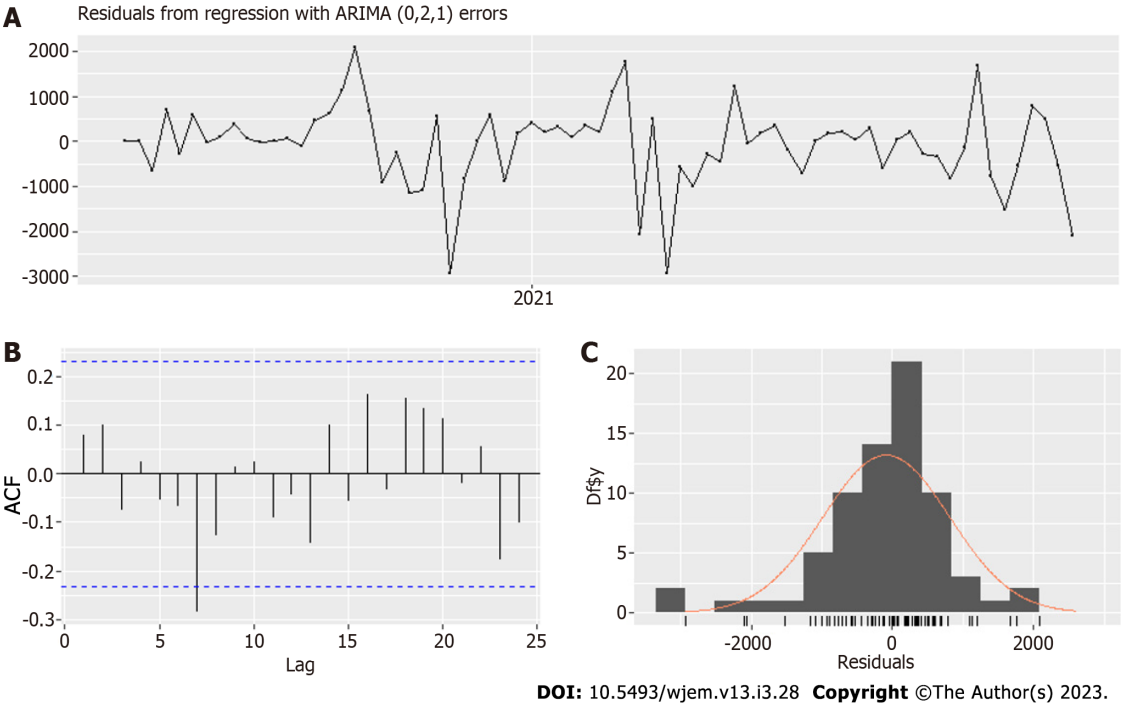

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Figure 7 Residuals of MODEL III –III – Temporal spread across age groups 20-49. A: Residuals of the model; B: Autocorrelation function of the residuals; C: Distribution of the residuals.

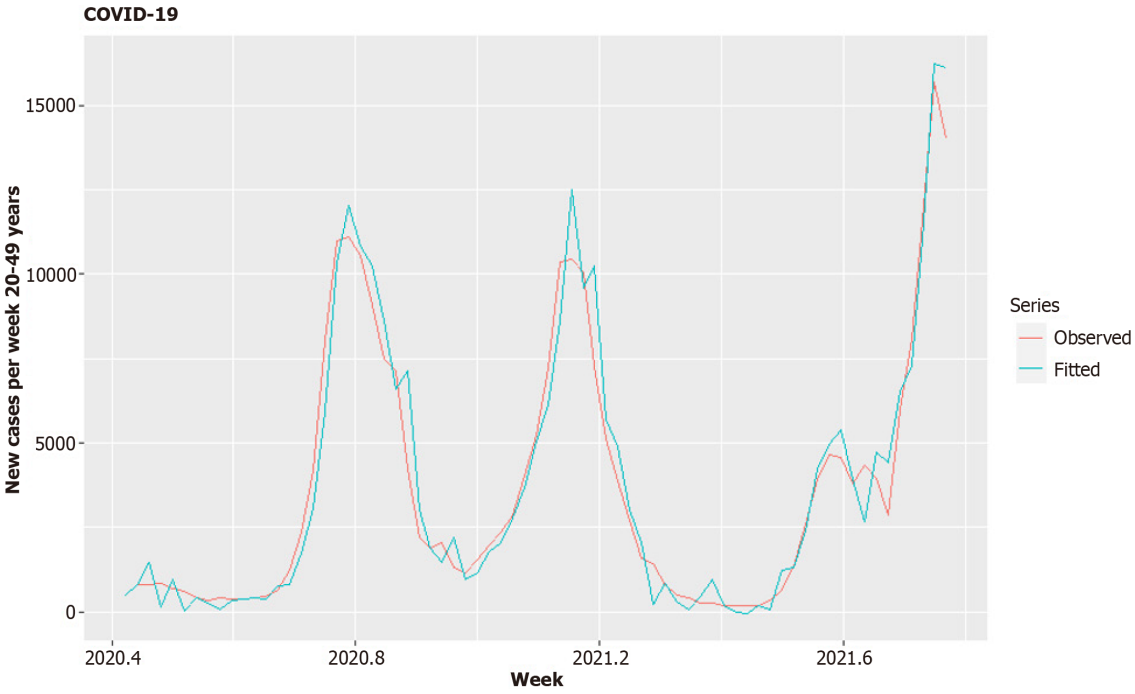

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Figure 8 Fitted MODEL III – Temporal spread across age groups 20-49. COVID-19: Coronavirus disease 2019.

Models II and III - temporal spread across age groups

For Model II and the age group of 60-89, the correlations (Table 3) are acceptable, and most of the coefficients are well estimated with small standard error, excluding variants (Table 4). The residuals are shown in Figure 7. The cases in the 0-19 group explain 96.2% of the variation in cases for the 60-89 years group (Figure 8).

The results for Model III showed that the new cases among 0-19 years old with a lag of two weeks are not necessary to predict the dynamics of the age group 20-49, and its removal improved the model. The correlations of this subset of regressors are the same as in Table 2. The model summary is given in

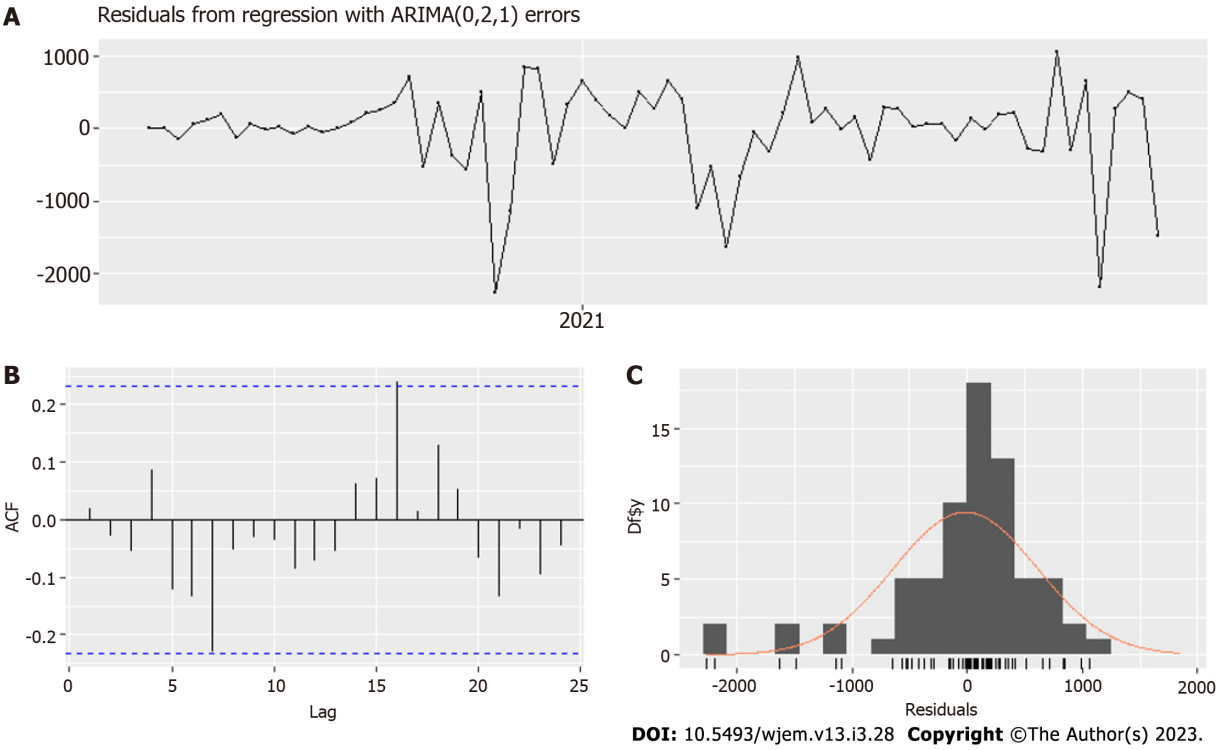

Figure 9 Residuals of Model II – Temporal spread across age groups 60-89. A: Residuals of the model; B: Autocorrelation function of the residuals; C: Distribution of the residuals.

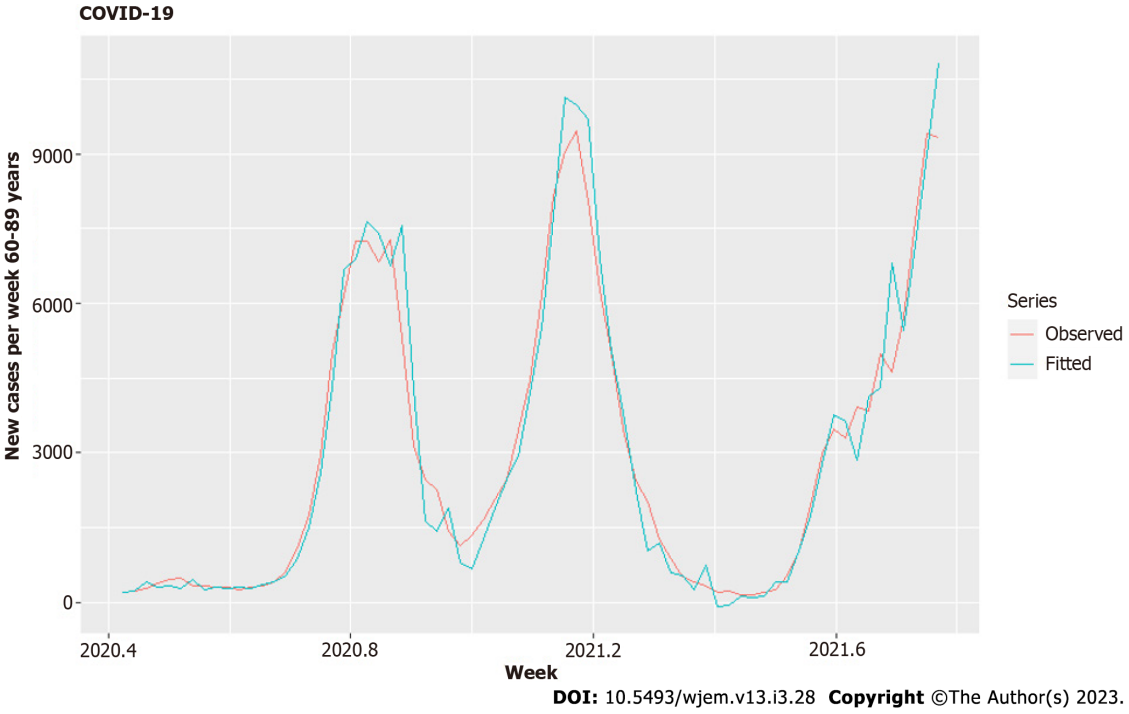

Figure 10 Fitted Model II – Temporal spread across age groups 60-89. COVID-19: Coronavirus disease 2019.

Table 5, the residuals are in Figure 9, and the fitted model is in Figure 10.

For the age group of 20-49 years, the influence of vaccinations in accelerating the spread among them is twice as strong – the coefficient in Table 5 is double relative to the same coefficient in Table 4 and with a relatively smaller standard error of estimation, bordering the proper ratio of 2:1 coefficient to error. One part of the explanation is that they are closely linked to children – the coefficient with Lag 1, which corresponds to the household infections is 5 times larger (a household effective reproductive number of 5.73 and the standard error of estimation is ten times smaller, than the error of the same coefficient in

the model for 60-80. The ratio of coefficient-to-standard error here jumps from 2:1 to 100:1. The role of the variant becomes probable in this age group – now, the standard error of estimation is smaller than the estimated coefficient. The current insufficient set of 74 observations increases the likelihood of coming to a positive conclusion for variants and spread among young people after the pandemic despite the acquired immunity of prior infections. Such a conclusion is also possible for the 0-19 age group, with a standard error of estimation close to the value of the coefficient, but is less likely for people in the 60-89 age group. It appears that the acceleration in the spread from variants is age dependent. One possible explanation is that with the increase of R0 of the variants, the age groups with more intensive contacts get more "advantage" over other age groups. Another explanation would be a saturation point in age risks from the pathogen – a weaker immune system that lowers the threshold for the effects of virulence (and R0) on the spread. This would mean that from some R0 and above, the spread cannot accelerate further for specific age groups. These hypotheses need further analysis and testing in future research.

Results for MIS-C dynamics and probabilistic modeling

Probabilistic modeling is a less precise way to predict or explain the dynamics than the time series analysis approach we employed in this article's first section. The distribution in Figure 11 shows us there is over a 5% chance of having 5 children per week, which gives a wide interval of new beds are needed weekly, and with the non-stationarity of the process, this has to be recalculated every week. With the increase in prevalence among children with new viral variants, it is possible $\lambda(t)$ to increase further, which means that we need to forecast it to do these recalculations. The estimating procedure will amplify the forecast error (fitting data to the Poisson distribution). Therefore, we prefer the time series analysis approach even for the small number of MIS-C cases.

Results for time series analysis of MIS-C cases

Results for MIS-C are promising but not yet conclusive. We need larger datasets to which to apply our methodology to have a better prediction on a weekly basis. We consider this an extreme test of our approach – can we model the MIS-C dynamics from such a small set? It appears that we can. In our model, we try to regress the new MIS-C cases against the new cases for the age group 0-19 years (we don't have detailed data by smaller sub-groups for the entire pandemic).

Our model in Table 6 is for biweekly cases. Coefficient NC L21, which is with a lag of one biweekly period, or up to 14 d, is well estimated with a value to standard error 2:1. We have close to good evaluation for NCL22 (which is with a lag of two periods, or up to 4 wk) with ratio 1.58. For a good assessment of NC L23, we need more data. The significant lags allow us to model properly only after half of the period of 78 wk, so we consider these results as promising, not conclusive. However, even with that small data set, we can give some predictions with Model IV (Figures 12 and 13) and some explanation of the data variation. We will continue the effort to model these cases by expanding our dataset in joint research with other hospitals in Bulgaria.

DISCUSSION

We aimed to assess the role of children in the ongoing epidemic in Bulgaria and to predict the impact of the pandemic on MIS-C cases in Pirogov. The initial claims in the literature that children have no impact and schools do not need strict measures do not hold in our analysis. Furthermore, it is clear that children are drivers of the epidemic in Bulgaria, probably due to the few school measures and no quarantines for their families when a class is quarantined[31].

The role of children in the Bulgarian COVID-19

We were able to predict cases in two age groups that are societally linked to children – 20-49 and 60-89 – parent and grandparents (and their close same-age contacts). Even before modeling, a large lag between cases in children (0-19) and cases in these groups was visible (Figure 1). What brings us more information is the difference between these two groups and the age groups that are less likely to be connected in the same household with children – 50-59 and 90+. As our analysis shows, school closures were a vital part of the containment of previous waves[32,33]. For that reason, not only children with their intense contacts but children *via* schools were drivers of the pandemic waves up to and including Delta[34]. Although there are summer waves from children to adults (2020 and 2021), they are transient and reflect migrations from cities to villages and tourist destinations, while autumn and winter waves show stable trajectories while schools are opened and turn after the closures – which our model I captured.

The counterintuitive role of vaccinations

We can see some plausible counterintuitive influences of vaccinations on the spread among adults in 60-89 years. It appears to have a strong positive impact, accelerating the spread (of course, for such conclusions, more research is necessary). The standard error of estimation is smaller than the coefficient. It is not small enough to accept the value of the coefficient but enough to give us some confidence about

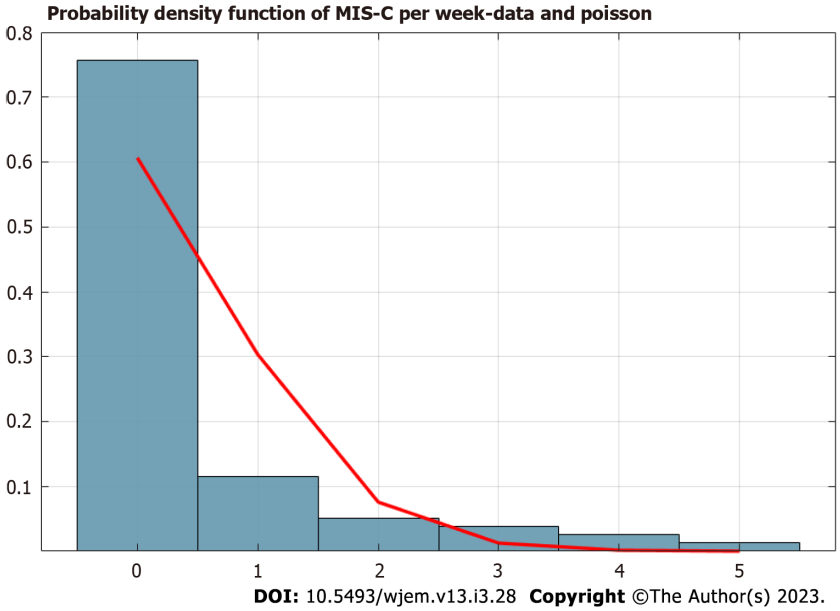

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Figure 11 Probability density distribution of the number of multisystem inflammation in children cases on a weekly basis. Poisson distribution for total cases up to 4.12.2021 is with λ = 0.50∈ [0.355, 0.835]. MIS-C: Multisystem inflammation in children.

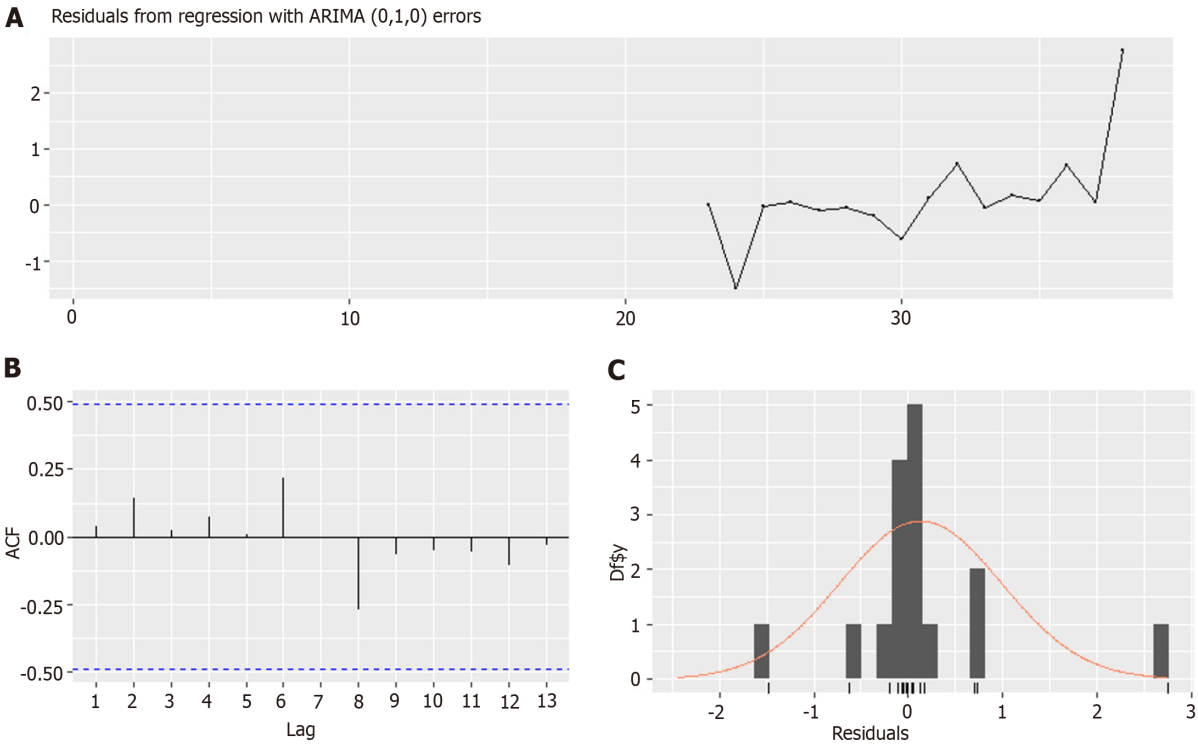

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Figure 12 Residuals of model for the bi-weekly period. A: Residuals of the model; B: Autocorrelation function of the residuals; C: Distribution of the residuals.

the direction of the influence. The current vaccination policy in Bulgaria, which is not focused on risk groups, does more harm than good since the mortality risk grows exponentially with age[16]. What is the reason? At first glance, the paradoxical negative impact of the vaccination program on increasing the incidence of COVID-19 can be explained by the rapid relaxation of anti-epidemiological measures at a deficit of vaccination coverage. The delay according to the model of the MIS-C case by 1 year is interesting because right now we have such a wave, one year after the previous one."

Furthermore, vaccines accelerate the spread significantly among children, as our first model showed, and as our second model demonstrates, the spread among 60-89 is strongly predicted with lags of 1, 2

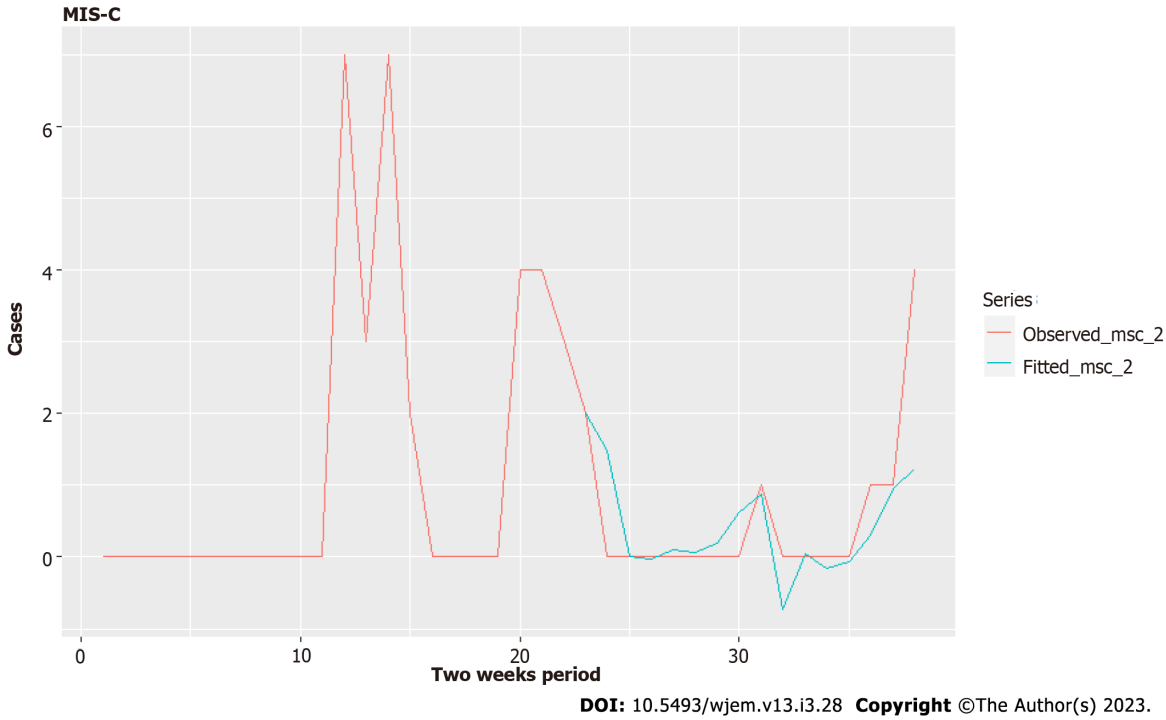

Figure 13 Fitted model for a bi-weekly period. MIS-C: Multisystem inflammation in children.

and 3 wk from cases in the 0-19 age group. The lack of exponential distribution of vaccinations across age groups (Figure 14) that would match the risk probably leads to a net negative effect on the number of cases among older individuals. Currently, the low vaccination rate translates this negative effect into a negative impact on deaths, which can be estimated from our model for the age-mortality risk. This might change with an increase in vaccination coverage or a change in the distribution of vaccination coverage.

CONCLUSION

Our modeling and data show with high probability that in Bulgaria, with our current measures, vaccination strategy and contact structure, the pandemic is driven by the children and their contacts in school. This is the simplest explanation of our three models. Furthermore, the lack of vaccination strategy accelerates the spread among children and from them to the age groups linked to them. For a country with low vaccination coverage of 23% up to November 16, 2021, this translates into an increased number of deaths and hospitalizations. There is a possibility of emerging variants to accelerate their spread among people below 50 years, thus compensating for the effect of acquired partial herd immunity, but that has to be confirmed by a larger set of observations and possibly new models to catch precisely the effects of the variants. This hypothesis and the other two we outlined as reasons for this age-restricted acceleration of the spread are part of our future research, as long as updating these three models after the end of the delta wave.

The modeling of MIS-C cases comes with few surprises – first, it is possible to model it with ARIMA even for a very low case amount, only for Pirogov, not on a national level. Suppose we put together all cases in Bulgaria. In that case, we will have a substantially larger dataset and the ability to make practically usable forecasts for MIS-C based on the historical record. Second, the optimal lags for prediction are huge – 41 to 48 wk or 21 to 24 bi-weekly periods. This is due to the low probability of occurrence of MIS-C among children or data artifacts (in which case it will change with a larger dataset). Third, the Poisson distribution can be used for modeling the probability of n cases arriving at a given week, but this is not a surprise, considering the independence of cases from one another in time. A challenge for modeling will be the arrival of new variants such as Delta, Delta+ and Omicron, which can change this probability and make historical data less useful for prediction. For that reason, we need the full dataset of MIS-C to model the dependency between the cases and the variants, which is part of our plan for future research, as well as modeling the network of covid-19 spread across ages in more detail, with more data for 0-19 subgroups (it appeared for first time in 25.09.2021 in the open data portal[35,36] and more elaborate dynamic network models).

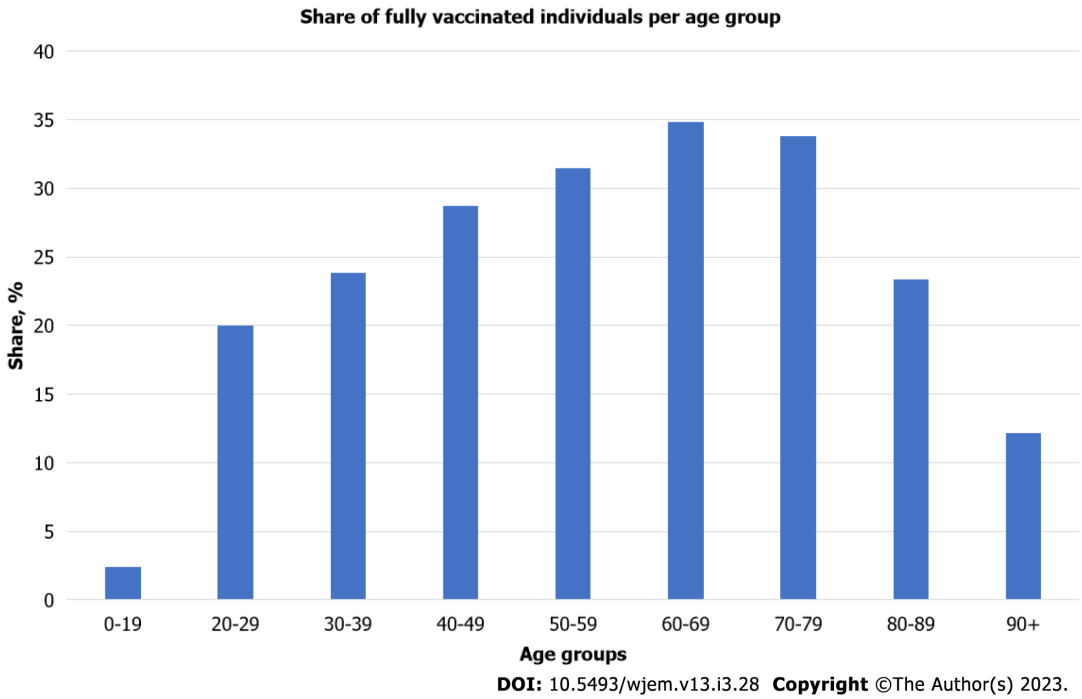

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Figure 14 Share of fully vaccinated individuals per age group up to November 16, 2021. Data for the number of fully vaccinated individuals is taken from[35].

ARTICLE HIGHLIGHTS

Research background

Transmission from children to adults was deemed secondary to the transmission from adults to children. After the first big wave in September to December 2020, masks were mandated in grades 5 and above but not enforced, with arguably low adherence, less than the general adherence due to mask removal in breaks between classes.

Research motivation

With starting the pandemic in Bulgaria, children were mostly speared from infection. On the one hand, it was thought that coronavirus disease 2019 (COVID-19) took a mild course in children. On the other hand, the testing was at the lowest rate among children. Up to now, the total number of infected children (0-18 years) is 103743.

Research objectives

We aimed to demonstrate the role of children in the COVID-19 spread in Bulgaria and to test the hypothesis that there are no secondary transmissions in schools and from children to adults.

Research methods

We used ARIMA models with external regressors and Poisson distribution modeling to test our hypotheses.

Research results

Our models allows to predict the new weekly cases in different age groups from the new cases in children one and two week prior. The age groups 50-59 and 90+ are less predictable and are also more isolated from children, consistent with the idea that children drive the pandemic in Bulgaria.

Research conclusions

Our models support the hypothesis that children are drivers of the pandemic in Bulgaria.

Research perspectives

In the first year of the pandemic it was suggested that children rarely transmit the virus to adults. On this basis Bulgarian Ministry of Health decided to allow the parents of quarantined children to continue to work and move freely in society. We will use more sophisticated models such as branching process to demonstrate that even if the probability of infection from children to adults is very low, the free

movement of the parents will still trigger a pandemic wave.

FOOTNOTES

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CORRECTION

Correction to "Performance of a serological IgM and IgG qualitative test for COVID-19 diagnosis: An experimental study in Brazil"

Fabrício Freire de Melo, Lilian Martins Oliveira Diniz, José Nélio Januário, João Fernando Gonçalves Ferreira, Rafael Santos Dantas Miranda Dórea, Breno Bittencourt de Brito, Hanna Santos Marques, Fabian Fellipe Bueno Lemos, Marcel Silva Luz, Samuel Luca Rocha Pinheiro, Gifone Aguiar Rocha, Dulciene Maria de Magalhães Queiroz

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Abstract

Correction to "Freire de Melo F, Martins Oliveira Diniz L, Nélio Januário J, Fernando Gonçalves Ferreira J, Dórea RSDM, de Brito BB, Marques HS, Lemos FFB, Silva Luz M, Rocha Pinheiro SL, de Magalhães Queiroz DM. Performance of a serological IgM and IgG qualitative test for COVID-19 diagnosis: An experimental study in Brazil. *World J Exp Med* 2022; 12(5): 100-103 [PMID: 36196438 DOI: 10.5493/wjem.v12.i5.100]". In this article, we identified an issue with the "Acknowledgments" section. Here, we then provide a recognition section for our supporting institutions.

Key Words: Correction; COVID-19 serological testing; Serologic tests; Rapid diagnostic

tests; SARS-CoV-2; Published erratum

Core Tip: This manuscript is to provide an "Acknowledgments" section to "Freire de Melo F, Martins Oliveira Diniz L, Nélio Januário J, Fernando Gonçalves Ferreira J, Dórea RSDM, de Brito BB, Marques HS, Lemos FFB, Silva Luz M, Rocha Pinheiro SL, de Magalhães Queiroz DM. Performance of a serological IgM and IgG qualitative test for COVID-19 diagnosis: An experimental study in Brazil. *World J Exp Med* 2022; 12(5): 100-103 [PMID: 36196438 DOI: 10.5493/wjem.v12.i5.100]".

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FOOTNOTES

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ACADEMIC WRITING

Writing strategies for improving the access of medical literature

Pratishtha B Chaudhari, Akshat Banga

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Abstract

When conducting a literature review, medical authors typically search for relevant keywords in bibliographic databases or on search engines like Google. After selecting the most pertinent article based on the title's relevance and the abstract's content, they download or purchase the article and cite it in their manuscript. Three major elements influence whether an article will be cited in future manuscripts: the keywords, the title, and the abstract. This indicates that these elements are the "key dissemination tools" for research papers. If these three elements are not determined judiciously by authors, it may adversely affect the manuscript's retrievability, readability, and citation index, which can negatively impact both the author and the journal. In this article, we share our informed perspective on writing strategies to enhance the searchability and citation of medical articles. These strategies are adopted from the principles of search engine optimization, but they do not aim to cheat or manipulate the search engine. Instead, they adopt a reader-centric content writing methodology that targets well-researched keywords to the readers who are searching for them. Reputable journals, such as *Nature* and the *British Medical Journal*, emphasize "online search-ability" in their author guidelines. We hope that this article will encourage medical authors to approach manuscript drafting from the perspective of "looking inside-out." In other words, they should not only draft manuscripts around what they want to convey to fellow researchers but also integrate what the readers want to discover. It is a call-to-action to better understand and engage search engine algorithms, so they yield information in a desired and self-learning manner because the "Cloud" is the new stakeholder.

Key Words: Medical Subject Headings; Key words; Search engine optimization; Access; Citation; Impact factor

Core Tip: Reputable journals like *Nature* and *British Medical Journal* lay emphasis on 'online search-ability' of articles in their author guidelines. This article urges medical colleagues to 'look inside-out' when drafting manuscript – to not only draft manuscripts around what we want to tell fellow researchers, but rather draft it in such a way that it embeds well what they are looking for. We hope that following these best practices will make it easier for search engines to crawl, index, and understand your articles to present them higher on the web-based-search results. Employing these strategies are often about making small modifications to the manuscript.

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INTRODUCTION

The famous joke, *"Where should you bury something that you do not want people to find? - On the second page of Google,"* carries an important message for medical researchers on what the authors need to pay close attention to. Studies show that nearly 75% of users never scroll past the first page of search results when searching for general information on Google[1]. Additionally, the first position on Google's organic search results has a 32% click-through rate[2].

In medical literature, when performing a literature review for draft manuscript "A", the usual sequence of actions involves searching for keywords within bibliographic databases or search engines like Google. After selecting the most relevant article "B" based on the title's relevance and reading the abstract's content, they download or purchase the article and cite it in their manuscript "A." In this process, three major elements drive the reader to ultimately cite the article "B": The keyword, the title, and the abstract. This indicates that title, abstract, and keywords are "key dissemination tools" for research papers. If these three elements are not determined judiciously by authors, it may adversely affect the manuscript's retrievability, readability, and citation index, which can negatively impact both the author and the journal.

By comparison, when an author writes an article "B" that deserves to be cited in another article "A," their efforts are mainly focused on conducting a literature review, ensuring a content flow, and meeting the target journal's manuscript formatting requirements. As a result, "keywords", "title," and "abstract" are often considered the last steps in this exhaustive process and are sometimes trivialized or obliviously determined while submitting the manuscript in the journal's portal.

The success of the author is typically measured by the number of manuscripts they have authored and the number of citations those articles have received. However, for an article to be cited, it must be retrievable by readers through a search engine[3]. It is evident from the above comparison that the medical community has not yet fully explored the potential offered by search engines to rank higher for specific search terms within the vast expanse of the internet and thereby enhance access to medical literature. This discrepancy between the approach of embedding keywords when an author drafts an article versus when a reader searches for it is illustrated in Figure 1.

In this manuscript, we share our informed perspective on writing strategies that can enhance the searchability and citation of medical articles. These strategies are adapted from the principles of search engine optimization (SEO) in content marketing. We do not intend to cheat or manipulate the search engines but rather adopt a reader-centric content writing methodology that embeds well-researched keywords that are targeted towards those readers who are searching for them, thereby enhancing the article's discoverability and reach. The central theme of this article is to encourage medical authors to shift their perspective and "look inside-out" when drafting manuscripts. In other words, they should not only draft manuscripts around what they want to convey to fellow researchers but also integrate what the readers want to discover. Within this manuscript, we describe the medical article indexing systems and propose strategies for enhancing the retrievability of published articles across search engines. A tabular comparison with the non-medical content writing strategies of SEO is also included.

SETTING THE BACKDROP: WHAT ARE THE MEDICAL MANUSCRIPT INDEXING SYSTEMS?

For brevity, the indexing systems employed by PubMed and Google are discussed here.

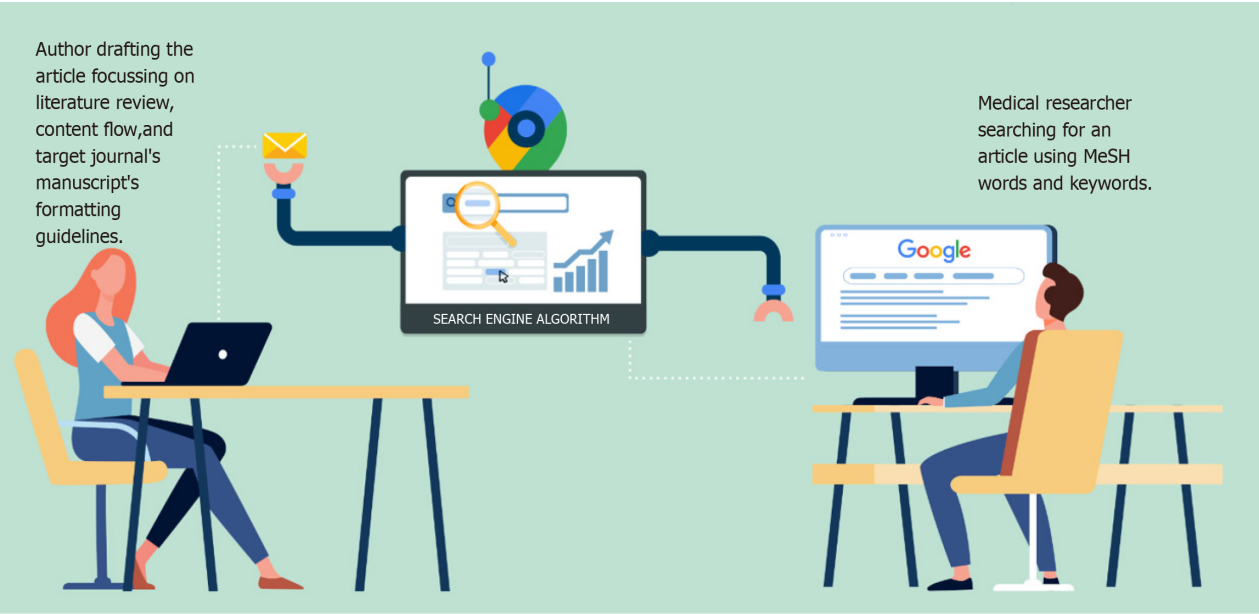

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Figure 1 The discrepancy between the approach of embedding keywords when an author drafts an article *vs* when a reader searches for it.

PubMed

PubMed is a web-based resource that provides free access to research publications. It is an excellent first choice to review medical research topics, as it indexes over 5000 journals and nearly 30 million journal article references, covering all of the pre-clinical sciences and biomedicine. Phrases in PubMed are recognized through the subject translation table used in the system's Automatic Term Mapping. For example, if we enter "fever of unknown origin," PubMed recognizes this phrase as a Medical Subject Headings (MeSH) term. The National Library of Medicine (NLM) created a controlled, pre-defined, hierarchically organized vocabulary called the MeSH thesaurus, which is used for searching, indexing, and listing biomedical and health-related information. MeSH includes the subject headings appearing in MEDLINE, PubMed, the NLM Catalog, and other NLM databases. When standardized terms are used to search a topic, all the articles indexed in NLM's PubMed and MEDLINE are retrieved, resulting in an increase in citations for the article. Controlled vocabularies are systematic, hierarchical arrangements of words and phrases designed to describe and categorize the major subject concepts and conditions contained within a database. They can be different for different databases. The hierarchical nature of these lists narrows the search to fewer yet more specific results, keeping it consistent within that framework. Before adding an item to a database or catalog, the subject matter is determined, and specific terms that apply to those subjects are chosen from a predetermined list, regardless of the terminology used by the author within the item. The listing is standardized and predictable to an extent, ensuring a uniform system for retrieving the same concepts even when different terminology is used. For example, the term "heart attack" is always listed as "myocardial infarction" within a controlled vocabulary structure such as MeSH, the vocabulary used by MEDLINE. The disadvantages of MeSH are enumerated in Table 1.

PubMed uses a hierarchical structure to display MeSH terms, which includes broader and narrower "descriptors." The top level of the MeSH tree structure consists of 16 broad categories, which are not included in the MeSH data maintained and distributed by NLM. However, they can be used to search PubMed by using the search term "category." For example, searching for "anatomy category" will retrieve all citations indexed under any MeSH descriptor in the "A" category (anatomy). When using a MeSH descriptor to search, PubMed automatically searches for narrower descriptors listed under it in the MeSH tree structures. For example, searching "musculoskeletal neoplasm non-Hodgkins lymphoma" will show the following two trees displayed with catalog numbers called tree numbers.

Each article citation is associated with a set of MeSH terms that describe the content of the citation. When conducting a literature search, using MeSH entry terms instead of keywords can result in a more focused search and make finding more relevant citations easier. For example, if we want to search "non-Hodgkins lymphoma", finding the corresponding MeSH term can help us narrow down our results. The actual MeSH term for this topic is "non-Hodgkins lymphoma," which is further subdivided into additional categories. Our search term may fall under multiple categories, such as neoplasms and immune system diseases, as shown in the example below. Sometimes, users may begin their search with a specific MeSH term; for example, they may start with "non-Hodgkins lymphoma," but then realize

Table 1 Disadvantages of Medical Subject Headings

Disadvantages of MeSH
1 MeSH terms are manually assigned to articles after an article is made available on PubMed. Manually assigned MeSH terms are not available for recently published articles. This is a time-consuming process, and hence, these articles fail to appear in search results when only MeSH terms are used for searching
2 In a scenario where no appropriate MeSH term is found while searching for a concept, it becomes important to search for relevant words in the title and abstract as well
3 Mesh search allows us to restrict our PubMed search to only find articles where the MeSH term is the main topic. In this way, articles in which the term and any selected subheadings are indexed as a major topic are displayed. For example, searching for "mechanical ventilation"[Majr] will exclude articles where this topic is covered to a lesser extent from the search results
4 Since PubMed only contains abstracts of articles, not full texts, searching the entire article for words is not possible with the use of MeSH
5 Sometimes, PubMed might fail to automatically match the exact MeSH term with the search
6 Additionally, some readers might not be comfortable using Boolean operators and logic. Boolean operators are not recognized by all databases, and the availability of all operators in a database might also be questionable
7 Moreover, databases also differ in their way of using syntax to enter a Boolean operator. For example, in Scopus, "NOT" is entered as "AND NOT."
8 Certain databases or search systems do not affect search results based on whether the terms are entered in uppercase or lowercase, but some engines like Google Scholar may require capitalized Boolean operators for proper functioning

MeSH: Medical Subject Headings.

they really want information on "lymphoma" in general. By using the MeSH tree structure, users can easily access a complete search on any term within the hierarchical tree with a single MeSH term.

Google

Google is the world's most popular search engine, which stores all web pages in its index. The content and location (URL) of a page are described by its index entry. The process of identifying new or updated web pages is called "Crawling." Google discovers web page locations (URLs) by many different means, including tracing links and reading sitemaps, among others. As Google crawls the web, it looks for new pages and indexes them when appropriate. A crawler is automated software that crawls (fetches) pages from the web and indexes them.

Google websites are evaluated for their medical reliability using the "Health On Net" or "HON" code. HON is a non-profit organization that promotes transparent and reliable health information dissemination online and acts as a quality marker for online health information. Regular assessments are carried out by medical experts with great vigilance to provide reliable information. The HON Foundation is a non-governmental organization officially related to the World Health Organization, which carries out certifications.

Google releases "search quality rating guidelines" periodically to ensure that search engines provide a diverse set of reliable, high-quality search results, presented in the most helpful order. Medical content falls under the category of "Your Money or Your Life" pages. This category includes all information that could potentially impact the future happiness, health, financial stability, or safety of the individual. Google is responsible for representing such information in the required indexing manner and has very high rating standards for such pages. When Google search quality raters evaluate a website, they are guided to look for expertise, authority, and trustworthiness, abbreviated as "EAT". Their ratings are further augmented by the algorithm, and that is when the information is displayed in a responsible manner.

WRITING STRATEGIES TO AUGMENT THE SEARCHABILITY OF PUBLISHED MANUSCRIPTS

Here are some strategies to use when creating the manuscript with the intent of ranking well in the search engines and reaching the relevant readers, ultimately yielding a higher number of clicks, downloads, and citations:

MeSH keyword gap analysis during the manuscript drafting phase

To increase the chances of an article being found and cited, we suggest conducting a "MeSH keyword gap analysis" at the beginning of the manuscript writing process. This approach can potentially improve the retrievability and citation of the article. Currently, most authors only determine and mention keywords during the manuscript submission stage without putting detailed thought into it,

which may result in missing out on strategic keywords.

Awareness of the journals and their role in keyword optimization

The wider reach of an article is not only important for the individual author but also beneficial for the journals. It helps the journals boost their impact factor, which is a scientometric measure of the frequency with which the average article in that journal has been cited in a particular year. The impact factor is used as a proxy for the importance or rank of a journal by calculating the number of times its articles are cited. Therefore, higher citation rates are beneficial for the ranking and credibility of journals, which implies they must focus on keyword optimization as well. The journals should hire professionals with an SEO background who are able to analyze the manuscripts for optimal use of keywords.

The list below complies the author's instructions for keywords for selected journals. Except for *Nature* and the *British Medical Journal* (BMJ), none of the journals mention the importance of searchability, page ranking, or SEO in their author guidelines. It is evident that currently there is not much emphasis on the optimized use of keywords, their selection, or placement. The only exception is *Nature* publishing house, whose journal author guidelines state that "*We ask authors to be aware of abstracting and indexing services when devising a title for the paper: providing one or two essential keywords within a title will be beneficial for web-search results*". They also suggest authors "*choose keywords to maximize visibility in online searches as well as being suitable for indexing services*"[4]. Meanwhile, the *BMJ* authors hub highlights that "*it is essential for your paper to be correctly set up for discoverability, right from the start*", and provides six steps to make the work more visible and, as a result, more likely to be cited.

Author instructions for keywords within a few sampled high-impact factor journals

New England Journal of Medicine[5]: Three to 10 keywords or short phrases should be added to the bottom of the abstract page, which will assist us in indexing the article and which may be published with the abstract. Use terms from the MeSH in *Index Medicus* when possible.

PLOS One[6]: Add keywords to help expedite the processing of your manuscript (optional). You will not have an opportunity to make changes, so make sure to add concise, accurate keywords now.

Journal of Clinical Oncology[7]: Immediately after the abstract, provide a maximum of six keywords, using British spelling and avoiding general and plural terms and multiple concepts (avoid, for example, "and" and "of"). Be sparing with abbreviations; only those firmly established in the field may be eligible. These keywords will be used for indexing purposes.

Annals of Internal Medicine[8]: No mention or instructions on keywords.

BMJ[9]: "*It is essential for your paper to be correctly set up for discoverability, right from the start*", and it provides some steps to make the work more visible and, as a result, more likely to be cited.

Keywords selection

Keywords play a crucial role in determining the discoverability of an article, leading to a chain reaction that includes clicking through, dwelling on, downloading, and ultimately citing the article. Citation is the critical element that generates the greatest impact factor. Keywords help readers find the best-suited article aligned with their research question and enhance the discoverability of research articles by ensuring that papers are substantially indexed by databases and search engines. It is important to select well-chosen keywords that best represent the article content and include various interchangeable terms and synonyms, be they in abbreviated or phrase form, making it easier for the manuscript to be easily identified and cited. The keywords of a manuscript should be selected by authors in such a manner that, if fed into search engines, accurate articles or the book's contents are retrieved. However, keyword selection can be subjective and differ amongst authors, leading to difficulty retrieving similar articles by authors who are searching the relevant literature for citations in their future articles. If keywords are used incorrectly, it may affect the citation index of the article. Therefore, it is a good practice to do a dipstick test by searching for some keywords in PubMed and analyzing the search results that reflect similar papers as ours. It is also essential to integrate the keywords well in the title and abstract, as they may not yield the article in the results if the manuscript is paid and the full text is not visible, as the search is based on the title and abstract.

Keywords are essential components of a manuscript and are typically listed after the abstract. Most journals require authors to provide three to five keywords, although some may ask for up to eight. Therefore, it is recommended to have eight to ten relevant keywords prepared before submitting the manuscript. Unfortunately, keyword selection is often left as the last step during manuscript submission, which can lead to an inadequate selection.

The problem with keywords is that they are not standardized because authors generate them, which cannot be exact words as in contents and can vary from author to author. Thus, they may not retrieve similar articles from different authors who are searching for the same relevant literature. Conducting effective MeSH term research and drafting the manuscript based on MeSH terms can increase online

Table 2 Search engine optimization strategies and corresponding adapted medical writing strategies

S. No.	SEO strategies	Corresponding medical writing strategies
1	Backlinks to share link equity	1 Citations of articles that enhance the credibility of articles 2 Blog about the article on forums like WordPress and link to it 3 Diversified media coverage, including social media articles linking the article, podcasts, and videos 4 Use opportunities to target different web areas like YouTube, podcast portals, social media, blogging portals, news, *etc.*
2	Organizing information into categories, pages, and subpages on a website and linking them to each other is called site architecture or information architecture	Have impactful headings using keywords within the headings
3	The flatter the site's structure is (requiring a lesser number of clicks to reach the desired information), the better its opportunity to rank well	MeSH terms and keywords search a manuscript by searching its abstract. Also, the abstract is commonly the only publicly available resource of an article, making it easily searchable for those on Google. Hence, incorporate critical keywords in the abstract of the article
4	The title tag is the most important on-page element to optimize for keywords, so the post has the best chance of ranking for a given keyword if that keyword appears at the beginning of the title tag (often the post headline). Also, make sure other posts linking to this one use this keyword as their link text (what SEOs call "anchor text")	Incorporate keywords in the title of the article. Give it some thought and brainstorm with the writing team about what keywords a potential researcher will search for. Use alternative terms and broader terms throughout the text to describe the concepts the authors are writing about because too specific terms may not filter in the search method
5	An average user looks at two things before opening a webpage: headlines and meta descriptions. The latter is always displayed below the webpage title in Google search results. Search engines highlight keywords in the meta description, thus making it a genuine call-to-action booster—it encourages people to click the link and check out the content	Meta description corresponds to the abstract of the article, particularly the objective and the initial three to four sentences
6	Carefully-designed keyword strategies to make more impact on Google's search engine algorithms	Research and integrate MeSH terms and keywords
7	Any time someone visits the website, we want them to have a positive user experience and engage with the site content	Lucid story-telling and point-to-point writing methodology
8	Visuals: when setting up pages, it is important to ensure that the pictures are always readable. "Alt" tags provide valuable details for the target audience. Google cannot understand images solely as image files	Explain the paper and integrate good-quality images and tables. Develop "at-a-glance" infographics covering key messages on the manuscript in a snapshot. Utilize digital designers, draw on easy digital tools like procreate, or hand draw and scan high-resolution images and convert them digitally to digitized images. Purchase editable vector images and templates. Determine figure legends judiciously, incorporating the keywords and MeSH terms, so their searchability is enhanced on Google Images
9	Internal linking is one of the SEO fundamentals that influence the dwell time of the target audience on the page	Generate the interest of the readers and direct them to various sections of the article by numbering the sections and structuring the topic well to enhance the time they spend on the page and article

MeSH: Medical Subject Headings; SEO: Search engine optimization.

traffic to the articles from the medical community more efficiently.

Research and use related search terms

Using synonyms and variations (related terms) of the keywords can help improve the discoverability of an article in the search results. For example, a reader looking for information on "heart attacks" may use search terms such as "heart attacks," "myocardial infarction," "myocardial infarctions," and so on. Therefore, when writing an article, it is important to embed various search terms that a researcher might use when searching for the relevant information that the article provides. Furthermore, it is important to pay attention to the terminologies that prevail in different geographies and how certain concepts and conditions are referred to based on medical, academic, and cultural backgrounds in that region. This information can be discovered through social listening and integrated into the manuscript. Although not yet widely practiced or emphasized, it is important to be mindful of MeSH keyword integration before and during manuscript writing. MeSH keywords may be searched, and all relevant words in the same hierarchy and those above and below it can be retrieved. It will help to identify keywords in the MeSH thesaurus that can be integrated throughout the article or abstract and title to perform well in the search engine algorithm.

Title and abstract

The ability to accurately highlight the core content of an article is crucial for crafting an impactful research paper title. A good title typically consists of a 10- to 12-word phrase with descriptive terms. Indexing agencies use the words used in the title to tag the article and make it easily discoverable. Making the title and abstract more action-oriented, concise, and less wordy can improve the search quality. Moreover, keywords provide an opportunity to tag the article with more relevant terms. Therefore, it is not a wise choice to use similar words or phrases both in the title and keywords.

The method of conducting a literature search differs from one person to another, which may result in different articles or a different number of articles despite looking for similar topics of interest. It is not always possible to predict how others will search the same literature. However, it is possible to elucidate how authors themselves might search the same literature. Authors should attempt to apply search strategies similar to those adopted by a reader or peer. Authors can utilize Google Trends to gain knowledge of internet search trends and discover the latest search trends for a particular word or phrase. Google Trends also helps in comparing search trends between different words and phrases, which helps authors choose the most relevant one for their title and keywords. From the perspective of the audience, study what types of topics the target audience would search for and that we would want our article to be found for and cited for.

Comparatively, an abstract is meant to provide a brief and accurate summary of the study, to aid the judgment of the reader of whether they should read the rest of the paper or not. A good understanding of the intent of the readers is foundational to this.

Effective visuals

Effective visuals help with readability and storytelling, greatly supporting learning, adding value, and keeping the reader engaged with the article. Vivid imagery is a much more effective and efficient method of communication than plain text. Articles that include images appear at the forefront of search engine results and have a viewership of up to 94% more often than those without images. Article visuals include tables, figures, clinical images, infographics, *etc*. Such elements can draw visual learners to our article. We can aid in the discovery process by making sure that our images and our site are optimized for Google Images. People who do not want to read an entire article but are still interested in the material can glance at these images and absorb valuable information without losing time. This helps engage and attract citations from readers who might have otherwise passed them by.

Images are proven to attract significantly more views compared to those without images, with a staggering 94% increase in views. Moreover, up to 60% of the population are visual learners, which means they prefer visual content over textual content. This is because the human brain is wired to process images faster than plain text, with images being processed 60000 times faster than text. Visuals are often available freely, even if an article is behind a paywall, and may even feature on Google Images, bringing in organic traffic to the article. It is important to carefully determine the figure legend and title by integrating the key MeSH terms. The average attention span is only around eight seconds, and visual content allows for valuable information to be distributed in a format that can be easily understood within this timeframe. In fact, according to the Massachusetts Institute of Technology, the human brain can process images in as little as 13 milliseconds. Good-quality visuals, developed with the support of professionals such as graphic designers, can lead to higher-quality traffic to the website by conveying valuable information in an engaging visual format. However, it is important to ensure that the visuals are original, as dealing with a copyright lawsuit can be an expensive affair.

Diversify the online visibility of the article

In addition to being an educational article intended for an academic and research audience, our manuscript will also be published as a webpage. A significant proportion of researchers and academicians search for articles not only on PubMed or MEDLINE but also on Google. Google attracts such traffic not only by analyzing words typed into its search box in a browser but also by using the words spoken to a mobile phone or assistant device and through "search engine auto-complete" features. This furthermore emphasizes the importance of the author's mindful efforts towards 'keywords and Google's searchability of them when drafting the manuscript.

To increase the article's online visibility, it is recommended to promote it through various channels, such as blogging, patient education, hospitals, and social media, along with linking the article. With educational content delivered in a conversational tone, our prospects get to know our target readers in a more personal way. Blogging is a particularly effective way to engage with readers, as more and more people are turning to blogs for information and sharing interesting articles on their social media networks. To leverage this behavior, it is crucial to cultivate the habit of blogging and maintain an active presence on all the relevant social media networks. Furthermore, some portals allow the authors to showcase unpublished, non-peer-reviewed articles that are ahead of print for the purpose of early clinical adoption and information sharing. However, it is only available for a limited time, mostly until publication. The SEO strategies and corresponding adapted medical writing strategies are enumerated in Table 2. Figure 2 showcases the proposed strategies for enhancing the searchability of manuscripts.

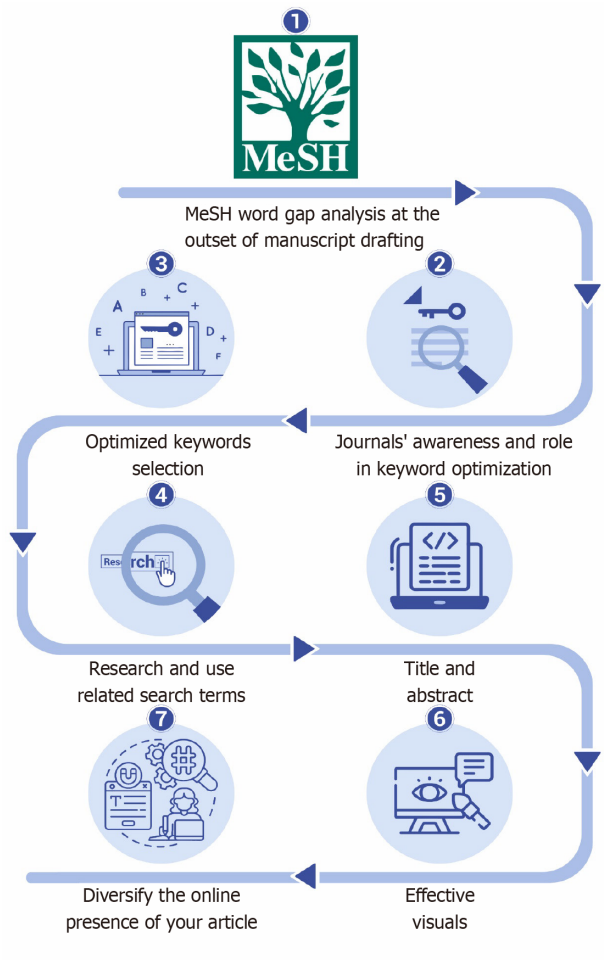

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Figure 2 Proposed strategies for enhancing the searchability of manuscripts—The art of being cited. MeSH: Medical Subject Headings.

LIMITATIONS

The discussion applies exclusively to the online readership, and we have naturally excluded from consideration those readers who read articles in journals in a hard copy format. The study assumed that clinicians would research information on their hand-held mobile devices and laptops in greater numbers, more so after the onset of the coronavirus disease-2019 pandemic. Similarly, information stored in textbooks is not included in this discussion. To some extent, Google Books information is included.

CONCLUSION

The medical community primarily searches for articles on PubMed using keywords and MeSH terms. However, not many authors write their articles with searchability in mind. Ideally, an article that answers a specific research question with a large sample size and rigorous research methodology should appear on the first page of the search engine. In reality, it is dependent on the accurate use of keywords, and one has to sift through dozens of articles to find the most well-matched article.

To enhance the visibility of an article, medical authors and publishers use various strategies, such as "open access availability of articles" and tagging articles in blogs and multiple media formats, including "podcasts, audio, and video formats." In addition to these existing practices, authors can improve the searchability of their articles by educating themselves about "Google keywords" and MeSH search and strategically embedding those words throughout the article. The point of this article is not to promote articles irrationally but to emphasize the importance of understanding the principles of search engine algorithms to enhance organic readership and uptake of the article.

We hope that following these best practices will make it easier for search engines to crawl, index, and understand the articles to rank them higher in the search results. Employing these strategies often involves making small modifications to the manuscript. When observed individually, these changes

might seem like incremental improvements, but when combined with other optimizations, they could have a noticeable impact on the article's performance in organic search results. The time has come that we understand and engage the search engine algorithms better, so they yield information in a desired and self-learning manner.

We acknowledge that the consumption of medical updates by healthcare professionals has become more digital in the past few years. Due to this, the updates we receive are also determined by a new 'factor' - the internet and its search engine which yields information based on the search words it is fed. Hence when drafting a manuscript this new factor must be considered along with technical accuracy of drafting research and grammatical flow. Hence not only the journal and the readers but also the "Internet" or the "cloud" is also a stakeholder in this process.

FOOTNOTES

Author contributions: Chaudhari PB and Banga A contributed equally to this work; Chaudhari PB and Banga A conceptualized the manuscript theme and outline; Chaudhari PB and Banga A performed the literature search and wrote the manuscript; All authors have read and approve the final manuscript.

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