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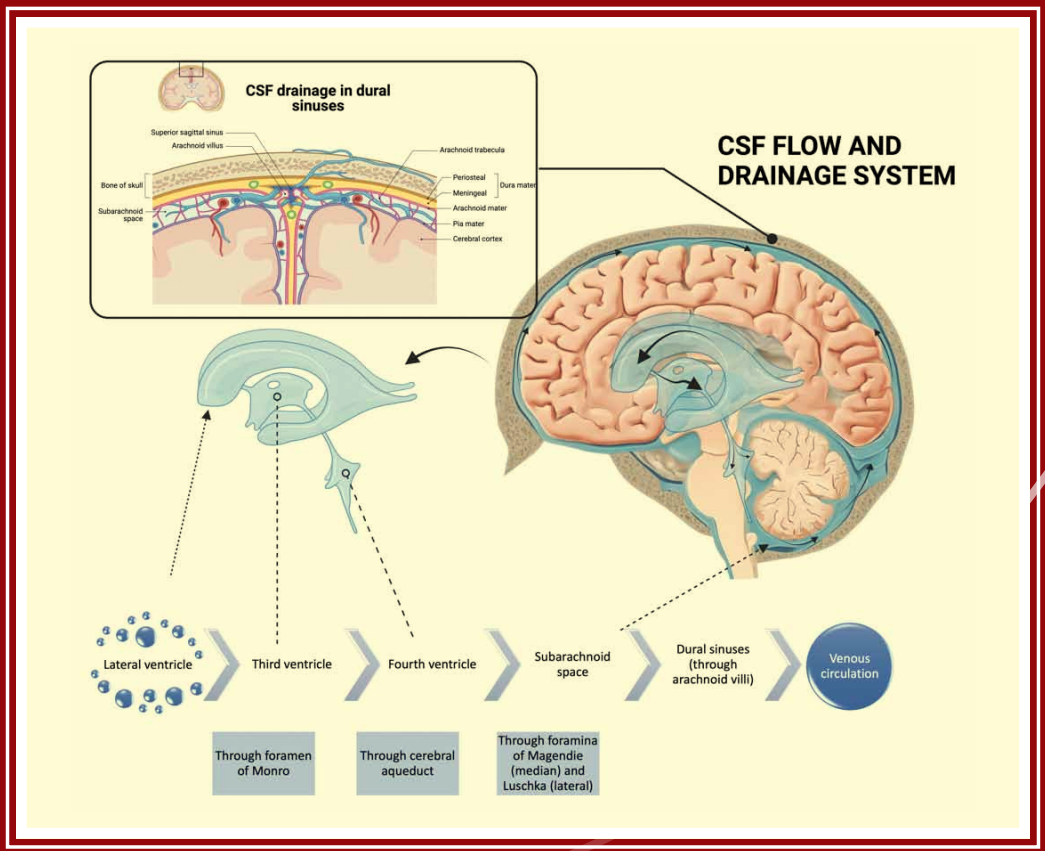

Schematic illustration of cerebrospinal fluid flow and drainage

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Dear colleagues,

In the current issue, the editorial by Lykouras et al. discusses the recent evidence on the management of chronic obstructive pulmonary disease (COPD) using triple inhaled therapy and provides data from clinical trials.

Moreover, this issue includes one original research article. The original research article by Kipourgos et al. assesses the knowledge, behavioral attitudes, and vaccination willingness among medical students in order to elicit valuable insights for the development of targeted educational initiatives, training programs, and public health campaigns. Lastly, this issue includes four review articles. The first review, by Papantoniou et al., investigates the importance of patient preparation and medical evaluation prior to endoscopic procedures, the potential need for medication dose adjustments, limitations in sedation, and strategies to ensure compliance with medical instructions. Additionally, it discusses the possible risks and optimal preparation for the two most performed endoscopic procedures, esophagogastroduodenoscopy (EGD) and colonoscopy and analyzes potential complications and safety measures associated with other endoscopic procedures. The review by Papageorgiou et al. explores the range of mechanisms employed by infectious diseases, which contribute to the occurrence of T-cell immunodeficiency. The review by Papanikolaou et al. provides an overview of the existing evidence regarding abbreviated double antiplatelet therapy duration with a focus on the management of patients with specific comorbidities, anatomical and technical issues during percutaneous coronary intervention. Lastly, the review by Katagi et al. demonstrates the genetic and molecular aspects of human hydrocephalus associated with congenital disorders and other complex pathologies including common syndromes such as primary ciliary dyskinesia and Dandy-Walker malformation.

Yours sincerely,

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Triple inhaled therapy in chronic obstructive pulmonary disease. Who is it for?

Dimosthenis Lykouras, Kyriakos Karkoulias

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) has become the third leading cause of death in the world at the beginning of this decade [1]. COPD management aims at relieving daily symptoms, improving exercise tolerance and quality of life. The prevention of exacerbations is an important goal of COPD treatment as well, because they are linked to disease progression and increased mortality. At the same time, management of the comorbidities of COPD, mainly cardiovascular, is crucial for keeping overall disease burden under control [2].

The Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommends the use of inhaled therapy with bronchodilators as initial treatment for the maintenance of stable COPD. The most commonly used are long-acting bronchodilators, namely long-acting muscarinic antagonists (LAMAs) and long-acting beta2-agonists (LABAs), alone or in combination. For patients with high blood eosinophil counts (>300 cells per μL), the addition of inhaled corticosteroids (ICS) is considered. Patients that suffer from frequent exacerbations or may have significant dyspnea in follow-up visits should also receive treatment with inhaled corticosteroids (ICS) together with bronchodilators. Currently, there are several inhaler combinations of these three pharmacological classes, including triple therapy in a single inhaler [3].

Clinicians often face the decision of whether to escalate treatment from a LABA/LAMA inhaler to triple therapy. Existing studies have compared the effectiveness and safety of different single-inhaler triple therapy options with single-inhaler dual bronchodilators, specifically LABA/LAMA, in COPD patients. Interpreting the data from these studies has been challenging.

Evidence from clinical trials

The IMPACT trial compared a single-inhaler triple therapy (umeclidinium, vilanterol, and fluticasone furoate) with two dual inhalers: a LABA/ICS (vilanterol and fluticasone furoate) and a LABA/LAMA (umeclidinium and vilanterol) over one year [2]. The trial included 10,355 COPD patients with moderate-to-severe airflow limitation and a history of exacerbations, including patients with asthma. Triple therapy showed a 25% lower rate of exacerbations and a 42% reduction in all-cause mortality compared to LABA/LAMA [4,5].

The TRIBUTE trial compared a single-inhaler triple therapy (glycopyrronium bromide, formoterol fumarate, and beclomethasone dipropionate) with a dual LABA/LAMA bronchodilator (glycopyrronium and indacaterol) over one year. The study included 1,532 patients with COPD having severe airflow limitation and at least one moderate or severe exacerbation in the previous year. Patients with asthma were included, as well in this study. All patients switched to the LABA/LAMA comparator after discontinuing their maintenance therapy during a 2-week run-in before randomization. Triple therapy arm of the study showed a 15% lower rate of moderate to severe exacerbations compared to the LABA/LAMA arm [6].

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Key words: *COPD; respiratory medicine; GOLD initiative*

The ETHOS trial investigated the effectiveness and safety of triple therapy in obstructive lung disease. It compared a single-inhaler triple therapy (consisting of glycopyrrolate, formoterol, and budesonide) with two dual inhalers: LABA/ICS (formoterol and budesonide) and LABA/LAMA (glycopyrrolate and formoterol). The study spanned over one year and involved 8,509 COPD patients with moderate-to-very severe airflow limitation and a history of at least one exacerbation in the past year. Some patients had a previous diagnosis of asthma. During a two-week run-in period, all patients discontinued their regular maintenance therapy and instead used short-acting bronchodilators. However, patients continued using ICS during the run-in and the use of ICS was randomly discontinued at the start of the trial. The results showed that triple therapy led to a 24% lower rate of moderate to severe exacerbations and a 46% lower mortality rate when compared to LABA/LAMA [7,8].

A real-world evidence study compared the effectiveness of single-inhaler triple therapy with dual bronchodilators in COPD patients. The study used data from the UK's Clinical Practice Research Datalink and included patients treated between 2017 and 2020. They looked at patients who had not used ICS but could have used LABA or LAMA. The study followed these patients for one year and adjusted for propensity score weighting to make the treatment groups comparable. The cohort included 4,106 new users of single-inhaler triple therapy and 29,702 users of dual bronchodilators. The results showed that triple therapy had a slightly higher risk of the first moderate or severe exacerbation compared to dual bronchodilators (adjusted hazard ratio of 1.08). However, triple therapy was more effective for patients with two or more previous exacerbations, existing asthma history, and high blood eosinophil count (>300 cells per μL). Triple therapy was associated with increased all-cause mortality and pneumonia [9].

Cardiovascular risk and expected benefit

There is evidence that inhaled maintenance therapy can help relieve symptoms, reduce exacerbations, and improve quality of life. The choice of therapy should be based on a comprehensive assessment of the benefits and risks for each individual patient, considering their specific characteristics [10]. COPD often co-exists with other complex health conditions, such as cardiovascular diseases, anxiety and depression, sarcopenia and peripheral muscle dysfunction, osteopenia and osteoporosis, lung cancer, anemia, or polycythemia [11].

Cardiovascular diseases are a significant cause of mortality in COPD patients, being second cause of mortality in those with mild-to-moderate airway obstruction, after lung cancer, and second cause after respiratory failure in those with severe airway obstruction. Common risk factors (age, smoking, pollution), systemic inflammation, reduced physical activity, lung hyperinflation, and hypoxemia contribute to the high prevalence of cardiovascular diseases in COPD patients. Exacerbations further increase the risk of cardiovascular events [12,13].

When evaluating the benefits and risks for individual patients, it is important to consider the impact of treatments on cardiovascular risk. The effects of inhaled COPD maintenance treatments on cardiovascular risk remain a topic of debate, with inconclusive evidence from available data.

A recent meta-analysis examined the occurrence of major adverse cardiovascular events in patients receiving combination therapy of LABA/LAMA with or without ICS. The study suggests that both treatment options involving dual bronchodilation increase the risk of cardiovascular events, particularly in patients with severe airflow obstruction. The yearly rate of cardiovascular events is estimated at least 1%. Additionally, the analysis showed that triple therapy (LABA/LAMA/ICS) resulted in fewer cardiovascular deaths than LABA/LAMA dual bronchodilator therapy, indicating a possible protective effect of ICS against cardiovascular events [14].

Withdrawing inhaled steroids

Another important study in the field is the SUNSET study, which examined the efficacy and safety of direct de-escalation from long-term triple therapy to indacaterol/glycopyrronium in non-frequently exacerbating patients with COPD. The results showed that in patients with COPD, but without frequent exacerbations on long-term triple therapy, it is possible to remove the ICS and de-escalate to LABA/LAMA with only a small decrease in lung function and no difference in exacerbations. The higher exacerbation risk was demonstrated in patients with ≥300 blood eosinophils per μl; thus, these patients are likely to benefit from triple therapy [15].

CONCLUSION

Although inhaled therapy can improve COPD prognosis through the reduction of exacerbations and improvement of lung function, inhaled treatments may

also cause adverse effects. Bronchodilator use is linked to increased cardiovascular risk and inhaled corticosteroids are associated with pneumonia and systemic side-effects.

Well-conducted observational studies can provide valuable real-world evidence that complements the major randomized clinical trials. Meanwhile, unbiased data analyses suggest that single-inhaler triple therapy should primarily be reserved for patients with frequent exacerbations, as described in GOLD suggestions. The majority of COPD patients should be given dual bronchodilators, which are proven to be equally effective treatment.

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ORIGINAL RESEARCH ARTICLE ACHAIKI IATRIKI | 2024; 43(4):166–175

The Role of Medical Students in Combating the Global COVID-19 Pandemic: Legacy for Future Health Crises

George Kipourgos[1], Georgios Vasilagkos[2], Angelikh Gkotsi[2], Evangelia Andreopoulou[1], Antonios Karanasos[2], Eleni Jelastopulu[3], Anastasios Tzenalis[4], Periklis Davlouros[2], Grigorios Tsigkas[2]

Abstract

Background: The global COVID-19 pandemic had a profound impact on societies, with healthcare professionals, including medical students, at the forefront of the battle against this infectious disease. Medical students, as a distinct subgroup, possess specialized knowledge and scientific background compared with the general population, making their attitudes and opinions crucial. Their knowledge, behavior, and adherence to preventive measures not only helped in curbing the virus's spread and safeguarding public health, but also influenced public opinion, offering reliable guidance to patients and countering misinformation.

Methods: A multicenter cross-sectional study was conducted among 466 medical students in Greece during the COVID-19 lockdown from January 15 to January 30, 2021. Participants were selected through snowball sampling from various academic institutions and completed an online questionnaire assessing their COVID-19-related knowledge, attitudes, and compliance to preventive measures.

Results: Among the participants, 78% demonstrated high knowledge levels about the virus and preventive measures, while 15% reported moderate knowledge, and only 7% had low knowledge. Regarding attitudes, 92% recognized the virus's severity and the need for adherence to preventive measures. Compliance was strong, with 89% strictly following mask-wearing guidelines and 78% avoiding mass gatherings. However, 21% admitted to occasional non-compliance. A strong correlation was identified between students' intention to vaccinate and their year of study, level of knowledge about the pandemic, and degree of compliance with preventative measures.

Conclusion: Medical students exhibited adequate knowledge about the virus, though improvement is needed in certain areas. Their positive attitudes and high compliance with preventive measures are encouraging. In addition, the findings showed an association between knowledge, adherence to restraints and the desire to vaccinate.

Key words: *COVID-19; Greek medical students; vaccination intention; restrictive measures*

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INTRODUCTION

The global COVID-19 pandemic has had profound repercussions on societies worldwide, placing healthcare professionals at the forefront of the battle against this infectious disease. Among these professionals, medical students undertook a pivotal role in comprehending and combating the virus [1]. As members of a distinctive age and cognitive subgroup, medical school students

possess specialized knowledge and scientific background compared to the general population, making their attitudes of paramount significance [2]. As future healthcare providers, their knowledge, behavioral attitudes, and adherence to preventive measures were critical in curtailing the virus's spread and safeguarding public health. They wield substantial influence on public opinion through their capacity to offer reliable guidance to patients, allaying their fears, and countering the dissemination of misinformation [3].

The acquisition of COVID-19 knowledge by medical students held particular relevance to their future roles as healthcare professionals. In the contemporary era, they have access to a myriad of credible scientific journals, online databases, and educational platforms that enable them to remain updated on emerging research and data related to COVID-19 [4]. Through continuous updates and medical education, they could acquire a comprehensive understanding of the virus, encompassing its modes of transmission, clinical manifestations, and preventive measures, thereby developing invaluable insights concerning the practical management of COVID-19 cases. Consequently, by acquiring knowledge about the coronavirus, they could become equipped to contribute effectively to ongoing efforts in combating it and providing optimal patient care [5].

Equally imperative was the investigation of medical students' attitudes and beliefs towards restrictive social isolation measures, as it was important to assess their adherence to recommended guidelines. Understanding their perceptions of preventive measures, such as social distancing, mask usage, and vaccination, can reveal potential gaps in their knowledge or misconceptions that may hinder the effective implementation of these measures in their future clinical practice [6].

Moreover, this article sought to explore the proportion of medical students who express willingness to be vaccinated against COVID-19. Vaccination plays a crucial role in preventing severe diseases, reducing transmission, and achieving herd immunity. Understanding the factors influencing their vaccination intentions is pivotal for public health strategies [7]. Identification of any concerns, hesitations, or misconceptions could aid in tailoring educational campaigns and interventions to promote acceptance and initiation of vaccination among medical students, and consequently, the general population.

In summary, this study endeavored to examine the knowledge, behavioral attitudes, and vaccination willingness among medical students. It aimed to elicit valuable insights that could contribute to the development of targeted educational initiatives, training programs, and public health campaigns. By empowering medical students with accurate information, fostering positive behavioral attitudes, and promoting vaccination, their preparedness could be enhanced, furthering more effective control and management not only of COVID-19 but also of future infectious disease outbreaks.

MATERIALS AND METHODS

Study Design and Participants

A multicenter cross-sectional study was conducted among medical students in Greece between January 15, 2021, and January 30, 2021, during the lockdown period imposed in response to the COVID-19 pandemic.

Participants were recruited using a convenience snowballing sampling approach. The inclusion criteria for the study required participants to be medical students studying at one of the country's public medical universities and possessing proficiency in reading and writing in Greek. Exclusion criteria mandated that participants not be registered medical students.

Data Collection

Data were collected using an online questionnaire specifically designed for this study. The questionnaire was developed by the authors following an extensive review of existing literature on COVID-19 and validated through a pre-testing process. The pre-test involved 50 medical students who were not part of the final study, and based on the results, modifications were made to the questionnaire to ensure its reliability and relevance.

The finalized survey instrument was adapted for administration via the "Typeform" online platform. The questionnaire comprised several modules, including:

Demographic and Anthropometric Data: This module gathered information on the participants' demographic characteristics and anthropometric measurements.

Cognitive Background COVID-19: Participants' knowledge and understanding of COVID-19 were assessed through a series of questions exploring their familiarity with the virus, its modes of transmission, and clinical manifestations.

Impact of the Pandemic: This module aimed to capture the perceived impact of the pandemic on medical students' lives, education, and mental well-being.

Level of Confidence in the Authorities: Participants were asked about their level of confidence in public

health authorities and government measures implemented during the pandemic.

Degree of Compliance with Measures During the Pandemic: This module investigated participants' adherence to preventive measures, such as social distancing, mask usage, and other containment strategies.

Intention to Vaccinate: The final module explored participants' intentions and attitudes towards COVID-19 vaccination.

Data Collection Process

The online questionnaire was distributed to eligible participants via email, newsletters, and social networks. Participants voluntarily completed the questionnaire through the "Typeform" platform, ensuring confidentiality and anonymity.

Statistical Analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 24.0 (IBM Corp, Armonk, NY, USA). Descriptive statistics for categorical data are presented as frequency counts and percentages. To assess the association between the dichotomous variable of intention to vaccination and other categorical variables of interest, the chi-square test of independence was employed. In instances where more than 20% of cells had expected counts below five, an asymptotic Likelihood Ratio Test was applied. Furthermore, proportions between the cells of two variables were compared using the Z-test. Variables associated with intention to vaccinate were assessed using ordinal regression, examining the intention to vaccinate as a semi-quantitative variable (No, probably no, probably yes, yes). Variables with a p-value <0.10 in the unadjusted analysis were used in the final multi-adjusted model. The threshold for statistical significance was set at $p < 0.05$, indicating that results with p-values below this threshold were considered statistically significant.

Ethical Considerations and Human Protection

This research adheres to the ethical principles set forth in the Helsinki Declaration (1964) and strictly follows the guidelines established by the European Network of Research Ethics Committees (ENREC) and the National Commission for Bioethics and Technoethics. The study's research protocol received approval from the Research Ethics Committee (R.E.C.) of the University of Patras. All study participants were provided with comprehensive information regarding the study's objectives, procedures, potential risks, and benefits, and they gave informed consent before participating.

Special emphasis was placed on safeguarding the privacy and confidentiality of participants' data. No personal identifying information, including names, emails, or IP addresses, was collected during data collection to ensure complete anonymity. Participants were explicitly assured of their voluntary participation and were given the freedom to withdraw from the study at any stage without incurring any repercussions.

The research team ensured diligent adherence to ethical guidelines throughout the study, ensuring the highest standards of research conduct and ethical responsibility in all aspects of the investigation.

RESULTS

Baseline Characteristics

The study involved a total of 466 medical students, with 37.1% being male and 62.9% female.

Regarding their distribution by year of study, the largest percentage of students (24.8%) were in their 2nd year of medical studies, followed by 20.5% in the 5th year. The remaining students were distributed as follows: 19.0% in the 3rd year, 14.7% in the 4th year, 9.8% in the 6th year, 5.6% in the 1st year, 2.8% in postgraduate studies, and 2.8% were graduates.

As for the university of study, the participants came from various academic institutions. Specifically, 45.1% of the students were from the University of Patras, while 25.6% were from the National and Kapodistrian University of Athens. Other universities represented in the study were: Democritus University of Thrace (11.3%), University of Ioannina (8.1%), Aristotle University of Thessaloniki (6.2%), University of Crete (1.5%), and University of Thessaly (2.1%).

Table 1 provides a concise overview of the participants' demographic characteristics, as well as their representation by gender and year of study. These indicators are important information for the sample profile and can enhance the understanding of the study findings.

Associations

The study employed a 10-question set to evaluate the participants' knowledge pertaining to SARS-CoV-2 and COVID-19, encompassing diverse aspects of the virus, preventive measures, and vaccination. Regarding the level of knowledge about COVID-19 outbreak, participants who scored 0-3 correct answers were categorized as having an "Insufficient knowledge level."

Table 1. *Descriptive overview of demographic data.*

Demographic Characteristics	n	%
Gender		
Male	173	37.1
Female	293	62.9
Year of Enrollment		
1st year	26	5.6
2nd year	116	24.8
3rd year	89	19.0
4th year	69	14.7
5th year	96	20.5
6th year	46	9.8
Postgraduate	13	2.8
Graduated	13	2.8
University of Enrollment		
National and Kapodistrian University of Athens	120	25.6
Democritus University of Thrace	53	11.3
University of Crete	7	1.5
Aristotle University of Thessaloniki	29	6.2
University of Ioannina	38	8.1
University of Thessaly	10	2.1
University of Patras	211	45.1

Those with 4-7 correct answers were categorized as having a "Moderate knowledge level." Finally, participants who scored 8-10 correct answers were categorized as having an "Adequate knowledge level." The majority of participants (68.8%) demonstrated a moderate level of knowledge, while a significant portion (27.6%) showed an adequate level of knowledge. A small percentage (3.6%) had insufficient knowledge (Table 2).

The results exhibited a noteworthy proportion of accurate responses in specific questions. Notably, a substantial majority of participants (95.9%) correctly identified the mode of virus transmission, while 91.9% displayed awareness that a person can yield negative test results shortly after infection. Moreover, 84.2% demonstrated a sound understanding that mRNA from vaccines cannot infiltrate the cell nucleus. Conversely, question 3 recorded the highest rate of incorrect responses at 65.2%. Merely 34.8% of participants were aware that a mother with a confirmed laboratory test for COVID-19 can safely breastfeed her baby. Table 3 breaks down all the relevant results for each question.

Table 2. *Cognitive background scale on COVID-19.*

Cognitive Background Scale	N	%
Insufficient knowledge level (0-3 correct answers)	17	3.6
Moderate knowledge level (4-7 correct answers)	322	68.8
Adequate knowledge level (8-10 correct answers)	129	27.6

Regarding the possible dependence of demographics and knowledge level, it was shown that none of the variables gender ($\chi 2=0.377$, p-value=0.828), year of study (p=0.313) and university of study (p=0.697) had a statistically significant effect. However, it was found that students from the University of Patras showed higher rates of adequate knowledge than other universities.

In addition, the survey results revealed the impact of the COVID-19 pandemic on the education and clinical practice of participants, as a significant majority (56.6%) reported a significant impact on their education, while only a negligible proportion (0.9%) reported no impact. Regarding perception of protection in the clinical setting, 36.5% felt adequately protected, while 14.7% expressed little to no sense of protection. Assessing the government's response, 64.7% believed that the Greek state moderately implemented appropriate measures to control the spread of the disease. Regarding the adequacy of the National Health System (NHS), only 3.4% considered it completely adequate, while 62.0% considered it moderately adequate. In particular, inadequate staffing of health structures emerged as the main handicap of the NHS in containing the pandemic (65.8%).

Another crucial aspect of the study was respondents' adherence to pandemic restrictions. The majority (60.5%) adhered and complied with the measures during both exclusions, while a particularly high proportion (34.4%) reported more compliance during the first exclusion. Importantly, a significant proportion (70.9%) confirmed that they did not falsely use the established exit codes during the first quarantine. In addition, the vast majority (94.2%) reported full compliance with isolation for 14 days after COVID-19 infection.

Regarding compliance, the results of the analysis showed that the majority of students demonstrated high compliance with protection measures. In particular, six questions were studied (Table 4). The researchers then categorized the results so that those who answered yes

Table 3. *Knowledge assessment questions on COVID-19.*

Question	Frequency	Percent
1. Select the main and MOST probable mode of transmission of the virus:		
Through the blood	1	0.2
Sexual intercourse	-	-
Droplets from the mouth or nose	**445**	**95.1**
From pets	1	0.2
From dead animals	2	0.4
Missing values	*4*	*0.9*
2. If someone gets infected, for how long can they infect others?		
7 days	56	12.0
14 days	**367**	**78.4**
I don't know	45	9.6
3. Can a mother with a confirmed laboratory test for COVID-19 breastfeed her baby?		
Yes, if he wishes. SARS-CoV-2 is not transmitted through breastfeeding	**163**	**34.8**
No. SARS-CoV-2 is transmitted through breastfeeding	71	15.2
I don't know	234	50
4. If you get infected with COVID-19 today, is it possible to have a negative test tomorrow or the day after?		
Yes	**430**	**91.9**
No	19	4.1
I don't know	19	4.1
5. Regarding the administration and performance of a Rapid test, the following apply (**multiple choice**):		
The Rapid test detects antigens (Ag) for the SARS-CoV-2 virus	**347**	**74.1**
The Rapid test detects two (2) of the genes of the SARSCoV2 virus	17	3.6
It is carried out while awake and taking a drop of blood	26	5.6
It is performed by taking a nasopharyngeal and/or oropharyngeal swab	408	87.2
They are mainly recommended for sample studies	304	65
They are mainly recommended for diagnosis and clinical decision making	36	7.7
I don't know	27	5.8
6. An efficacy of 95% for a vaccine implies that:		
Of those who became ill, 95% had received the vaccine	14	3.0
Of those who fell ill, 95% experienced adverse effects	2	0.4
Of those who became ill, 5% had received the vaccine	**260**	**55.6**
Of those who fell ill, 5% experienced adverse effects	143	30.6
I don't know	49	10.5
7. The immunity generated from COVID-19 vaccination lasts:		
For life	7	1.5
3 months	12	2.6
6 months	48	10.3

Table 3. *Knowledge assessment questions on COVID-19 (continued).*

Question	Frequency	Percent
Unspecified/Under investigation	**386**	**82.5**
I don't know	15	3.2
8. Appearance of fever and fatigue after receiving an mRNA vaccine might indicate:		
Correct	174	37.2
Wrong	**257**	**54.9**
I don't know	37	7.9
9. Can mRNA enter the cell nucleus, where our DNA is located?		
Correct	56	12.0
Wrong	**394**	**84.2**
I don't know	18	3.8
10. Immunity resulting from the actual disease is much better than that resulting from:		
Correct	55	11.8
Wrong	**275**	**58.8**
I don't know	138	29.5

Table 4. *Questions through which compliance to COVID-19 protection measures was assessed.*

Questions	Yes (%)	No (%)
1. Always wear a simple surgical or cloth mask indoors?	90.81	9.19
2. I wash my hands more often and/or more thoroughly.?	92.52	7.48
3. Disinfect surfaces more regularly and/or more thoroughly?	67.30	32.7
4. Avoid social gatherings and events?	89.95	10.05
5. Avoid shaking hands?	84.61	15.39
6. Avoid crowds and keep my distance?	92.52	7.48

Table 5. *Scale of compliance protection measures on Covid.*

Compliance Level	N	%
Insufficient (answered "yes" to 1-2 of the 6 questions)	20	4.3
Moderate (answered "yes" to 3-4 of the 6 questions)	67	14.3
Adequate (answered "yes" to at least 5 of the 6 questions)	381	81.4

to 1 or 2 of the 6 questions were considered to have Insufficient adherence to the measures, those who answered yes to 3 to 4 of the 6 questions as moderate adherence, and finally, students who answered yes to 5 or more questions were considered to have adequate adherence to the measures. The majority of students (81.4%) had adequate adherence to protection measures, while only a small number (4.3%) showed poor adherence (Table 5).

Uni- and Multivariate Analysis

The results revealed that gender significantly influenced the adherence level to protective measures ($\chi^2 = 8.294$, $p = 0.016$), with females exhibiting higher adherence. However, the year of enrollment ($\chi^2 = 21.052$, $p = 0.100$) and university attended ($\chi^2 = 7.745$, $p = 0.805$) did not show statistically significant associations. As to whether the degree of compliance was influenced by various factors, it was found that the variable "Have you been diagnosed with COVID-19 until today?", influenced the adherence level to protective measures during the pandemic. According to the results, this specific variable demonstrated a statistically significant association with adherence ($\chi^2 = 15.987$, $p = 0.014$). Specifically, individuals with a COVID-19 diagnosis displayed higher levels of adherence.

In terms of vaccination intention, the majority of medical students (73.1%) stated that they would be vaccinated, with a further 19.0% tending towards vac-

cination. Vaccine preferences showed that a significant proportion (82.9%) had a preference for mRNA vaccine over other types. Finally, medical students showed different opinions on when and how they would prefer to be vaccinated. A notable proportion (32.9%) would choose to be vaccinated at the same time as healthcare workers, with vaccination being mandatory. Similarly, 35.9% would opt for simultaneous vaccination with healthcare workers, but on a voluntary basis. In addition, 12.0% of students would prefer to be vaccinated at a later time and on a voluntary basis. Also, based on the data, there was no statistically significant association between demographic variables (gender, year of enrollment, university of enrollment) and the intention to vaccinate in the specific sample. On the other hand, the results showed that knowledge level may play a role in shaping vaccination intention, as individuals with a higher knowledge background, such as those categorized as having "Adequate" knowledge, are more likely to express vaccination intention compared to individuals with a lower knowledge background, such as those categorized as "Probably not" or "No" (Table 6). Similarly, the intention to vaccinate seemed to vary according to the level of protection measures. Individuals with a higher level of adherence (such as those in the 'Adequate' category) seem more likely to have an intention to vaccinate than those with a lower level of adherence (such as those in the 'Poor' and 'Moderate' categories) (Table 7).

Finally, after multivariate correlations, it was found that students' intention to vaccinate was influenced by both their level of knowledge about the pandemic ($p<0.001$) and their level of compliance with the measures ($p<0.001$). In contrast, vaccination intention was not shown to be influenced by year of study ($p=0.051$) (Table 8).

DISCUSSION

This study provides a detailed analysis of knowledge, adherence to restrictive measures, and the desire for vaccination in a sample of 466 medical students. The results reveal diverse aspects related to students' perception and response towards SARS-CoV-2 and COVID-19. Similar studies have been carried out around the world [8–16], highlighting the special role of medical students in the face of the pandemic.

Regarding demographic characteristics, the study observed that students were heterogeneous in terms of gender and year of study. The majority of the sample comprised female students, with a higher representation of students in their second year. The predominance of the female sex is confirmed in numerous similar studies internationally [17–21], while one study in India reported an equal gender ratio [22]. Additionally, the study included students from different universities, with the University of Patras having the largest representation.

Concerning students' knowledge, the majority

Table 6. *Correlation between the cognitive background scale on COVID-19 and vaccination intention.*

	Vaccination intention					
Cognitive Background Scale	Yes n(%)	Probably Yes n(%)	No n(%)	Probably No n(%)	Total n(%)	X2
Inadequate	9 (1.9%)	3 (0.6%)	2 (0.4%)	3 (0.6%)	17 (3.7%)	X2=63,720
Moderate	220 (47.4%)	68 (14.7%)	10 (2.2%)	21 (4.5%)	319 (68.8%)	<0.001
Adequate	110 (23.7%)	17 (3.7%)	0 (0.0%)	1 (0.2%)	128 (27.6%)	
Total	339 (73.1%)	88 (19.0%)	12 (2.6%)	25 (5.4%)	464 (100%)	

Table 7. *Correlation between the scale of compliance protection measures on COVID-19 and vaccination intention.*

	Vaccination intention					
Compliance Level	Yes n(%)	Probably Yes n(%)	No n(%)	Probably No n(%)	Total n(%)	X2
Insufficient	10 (2.2%)	5 (1.1%)	4 (0.9%)	1 (0.2%)	20 (4.3%)	X2=27,553
Moderate	38 (8.2%)	12 (2.6%)	4 (0.9%)	13 (2.8%)	67 (14.4%)	<0.001
Adequate	291 (62.7%)	71 (15.3%)	4 (0.9%)	11 (2.4%)	377 (81.3%)	
Total	339 (73.1%)	88 (19.0%)	12 (2.6%)	25 (5.4%)	464 (100%)	

Table 8. *Unadjusted and multi-adjusted analysis regarding vaccination intention.*

Unadjusted analysis		
Variable	Regression coefficient (95% confidence intervals)	p-value
Gender (male)	0.27 (-0.16 – 0.70)	0.22
Study year (per year of study)	0.19 (0.07 – 0.32)	**0.003**
Cognitive level (per level)	1.01 (0.56 – 1.46)	**<0.001**
Compliance level (per level)	0.84 (0.49 – 1.19)	**<0.001**
University		0.59
National and Kapodistrian University of Athens	0.49 (-0.04 – 1.01)	0.07
Democritus University of Thrace	0.09 (-0.57 – 0.74)	0.80
University of Crete	0.82 (-1.21 – 2.85)	0.43
Aristotle University of Thessaloniki	0.47 (-0.46 – 1.40)	0.32
University of Ioannina	0.05 (-0.70 – 0.79)	0.91
University of Thessaly	0.49 (-1.05 – 2.03)	0.53
University of Patras (reference)	0	
Multi-adjusted analysis		
Study year (per year of study)	0.13 (-0.00 – 0.26)	0.051
Cognitive level (per level)	0.93 (0.47 – 1.39)	**<0.001**
Compliance level (per level)	0.77 (0.41 – 1.13)	**<0.001**

demonstrated moderate knowledge, with a significant number showing adequate knowledge. Only a small percentage had inadequate knowledge. These findings align with previous studies [15,23–27], but contrast with the study by Amri et al [28].

Further analyzing the participants' understanding, the study found that students showed good adherence to restrictive measures, indicating their awareness of their pivotal role in containing the crisis. More than eight in ten students exhibited effective adherence to various restrictive measures. Similar moderate adherence was found in Tunisia [29], lower in two studies in Egypt [30,31], and higher in a study of nursing students in three European countries [32].

The study also explored the issue of the desire for vaccination and found that the majority of students stated they would be vaccinated. This finding aligns with other studies on medical students' attitudes towards vaccination in general [33] and with a similar study among doctors and nurses in Greece [34]. Understanding students' preferences and perceptions can inform public health strategies to promote vaccine acceptance and uptake among young adults and the broader population.

The discussion of the results highlights the need for further education and information for medical students in similar future health crises. Promoting adherence to containment measures and vaccination should be considered critical tools for controlling a pandemic. Educational programs can help improve understanding and acceptance of restrictive measures and vaccination, contributing to future public health protection.

The study also emphasized the importance of evaluating the impact of the pandemic on student education and clinical practice, a fact confirmed internationally by multiple research studies [35–38]. Identifying challenges faced by students and implementing appropriate measures can enhance the quality of their education and better prepare them for integration into the healthcare field.

Moreover, the findings on the associations between knowledge, adherence to restrictive measures, and the desire to vaccinate are interesting. Students with higher knowledge and adherence to restrictive measures tend to have a greater desire to vaccinate, as confirmed in a study by Alsoghair et al [23]. This suggests that students' understanding of the virus and preventive measures

may influence their decisions to vaccinate, leaving a lasting impact for the future.

Overall, the findings of this study provide important insights into medical students' perceptions and behaviors regarding SARS-CoV-2 and COVID-19. Understanding these aspects is critical for designing more effective disease prevention and control programs, as well as promoting responsible attitudes towards a pandemic. Efforts to improve knowledge, adherence to restrictive measures, and the desire to vaccinate can contribute to future public health protection and response to similar health crises.

It should be noted that this study has some limitations. Although the sample was quite large, the use of a larger and more representative sample could potentially lead to more conclusions. Additionally, responses may have been influenced by personal biases or socioeconomic factors not accounted for. Further research efforts should explore these factors for a comprehensive understanding of the results.

CONCLUSIONS

In conclusion, the present study analyzed knowledge, adherence to restrictions and vaccination willingness in a fairly large sample of medical students in Greece. The results revealed that most students demonstrated moderate knowledge of the topic and effective adherence to restrictive measures. It also clarified that the majority wished to be vaccinated, with 82.9% preferring mRNA vaccines. More specifically, individuals with a higher knowledge background as well as those who strictly abided by preventive measures, are more likely to express vaccination intentions. Interestingly, history of COVID-19 disease influenced the adherence level to protective measures during the pandemic. The study highlighted the need for further education and information for students in future health crises. Promoting adherence to containment measures and vaccination is essential to control a pandemic. Educational programs can improve understanding and acceptance of the measures, thus offering a contribution to public health by future physicians. Overall, the study is a valuable source of knowledge for dealing with similar crises in the future.

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Gastrointestinal endoscopy on elderly patients. Are there limitations?

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Abstract

Gastrointestinal endoscopy is increasingly performed in elderly patients for both screening and therapeutic purposes. However, this demographic presents unique challenges due to the higher prevalence of comorbidities and the widespread use of medications such as antiplatelets, anticoagulants, antidiabetic agents, and neurotropic drugs. Sedation related adverse events appear more frequently in elderly patients. Potential complications accentuated in the elderly include post intervention bleeding and perforation, dehydration and electrolyte imbalance following bowel preparation, post-cholangiopancreatography (ERCP) cholangitis, sedation-related adverse events and cardiopulmonary complications. Despite the established efficacy and safety of gastrointestinal endoscopic procedures, it is recommended that the decision to proceed with endoscopy should be individualized, taking into account factors such as expected benefits, performance status, cognitive function, understanding of potential complications, and adherence to medical instructions.

Key words: *Endoscopy; elderly; complications; preparation; individualization*

INTRODUCTION

The demographic shift towards an aging population has been a significant trend in recent decades, with individuals aged 65 and older being classified as geriatric patients [1]. In the European Union (EU), 21.1% of the population were aged 65 and over in 2022, compared to 18% a decade earlier, while in the United States of America (USA) people over 65 increased from 4.9 million (or 4.7% of the total population) in 1920 to 55.8 million (16.8%) in 2020 [2,3]. Cellular aging and physiological alterations as well as comorbidities and use of medications affect many gastrointestinal (GI) functions such as motility, response to hormone secretion and medication metabolism, thus leading to increased incidence of many GI diseases in the elderly. For example, reduced blood flow increases the incidence of peptic ulcers and mesenteric ischemia, while increased use of non-steroidal inflammatory drugs (NSAIDs) can cause stomach and small bowel bleeding [4]. Approximately 85.260 new cases of colorectal cancer were diagnosed in people over 65 in the USA for 2023 [5], while disorders such as diverticulosis, constipation and diarrhea are commonly observed in the elderly [6]. Many of these disorders can be prevented, diagnosed and sometimes treated with endoscopy.

Although endoscopy has demonstrated significant efficacy and diagnostic yield in elderly patients [7], ensuring the safety of these procedures is paramount. It is imperative to carefully assess whether the benefits of the procedure outweigh the risks of potential complications [8]. In this review, we explore the importance of patient preparation and medical evaluation prior to endoscopic

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procedures, the potential need for medication dose adjustments, limitations in sedation, and strategies to ensure compliance with medical instructions. Additionally, we will focus on the possible risks and optimal preparation for the two most commonly performed endoscopic procedures, esophagogastroduodenoscopy (EGD) and colonoscopy. Furthermore, we will analyze potential complications and safety measures associated with other endoscopic procedures.

PRE-ENDOSCOPY ASSESSMENT

Medical History and Physical Examination

Before conducting an endoscopy, it is crucial to perform a comprehensive history taking and physical examination. Assessing the patient's cognitive status and ability to provide consent is essential, and in cases where a patient lacks capacity, obtaining consensus from family members or caregivers may be necessary [9]. Given that modern endoscopy units implement devices that could potentially cause electrical interference, special attention should be given to patients with implanted devices such as defibrillators and pacemakers. Protective measures for both patients and staff before and during endoscopy should adhere to current guidelines [10,11].

Polypharmacy is commonly observed in older adults, necessitating the recording of all patient medications, including over-the-counter drugs. Close monitoring of diuretics, antihypertensives such as angiotensin-converting enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), and NSAIDs is essential, as they elevate the risk of dehydration and peri procedural hypotension [12].

Diabetes is a common comorbidity in the elderly. This patient population is at risk of complications during endoscopy preparation, including hypoglycemia, fluid and electrolyte imbalances, acute renal failure, lactic acidosis, and ketoacidosis [14]. Consultation with an endocrinologist for drug modifications is advisable. Morning procedures are recommended due to the heightened risk of hypoglycemia [14]. Patients should refrain from taking antihyperglycemics on the day of the procedure. Sulfonylureas and SGLT2 inhibitors should be discontinued the day before, and long-acting insulin doses should be halved, with glucose levels closely monitored. Normal treatment should be resumed at usual doses once the patient is permitted to eat and drink normally [13].

Antiplatelet and anticoagulant medications increase the risk of bleeding during and after endoscopy, particularly following high-risk procedures such as polypectomy, endoscopic mucosal resection, and sphincterotomy [15]. Patients should be informed about the risks associated with the procedure, and the appropriate timing for cessation and resumption of these medications should align with current guidelines. In high-risk cases, consultation with other specialties such as cardiologists and hematologists may be necessary [16].

Endoscopy indications

Like in any age group, endoscopy in elderly patients should only be undertaken when the results are likely to contribute to improved patient outcome [17]. Referring physicians need to consider if and how test results will affect patient management and care. While the indications and contraindications for GI endoscopy generally mirror those in younger adults, some variations exist due to the differing incidence of various GI diseases with advancing age [18]. It is important, however, to pay close attention to comorbidities that may increase the risk of complications, particularly cardiovascular and pulmonary dysfunction [17].

Bowel preparation

Achieving good bowel preparation is paramount for conducting a successful colonoscopy. However, this poses a challenge for the elderly population, who are less likely to tolerate high-volume regiment oral preparation [19]. Studies have demonstrated that older age is a risk factor for incomplete colonoscopy [20]. Diabetic patients are particularly susceptible to poor bowel preparation, often requiring repeat endoscopies [14]. Complications such as dehydration, electrolyte imbalance, increased risk of falls and fractures and acute renal failure have been observed during bowel preparation in older individuals, emphasizing the need for careful planning and selection of the appropriate type of oral preparation [21,22]. Magnesium-based solutions can lead to severe hypermagnesemia, even in the absence of kidney disease [23]. Sodium phosphate solutions may result in potentially fatal electrolyte disturbances such as hypokalemia and hyperphosphatemia, with their prevalence being higher in frail patients [24]. Polyethylene glycol (PEG) based solutions exhibit the best safety profile. They have been shown to be better tolerated and are therefore preferred for the elderly population [15,17]. Patients should be thoroughly informed about the risks of dehydration and potential kidney damage, as impaired renal fluid management is common in advanced age. Adequate hydration before and after

endoscopy is essential [14]. Split dosing, along with a low-fiber diet and proper timing of the endoscopy, appears to be an effective bowel cleansing strategy, as it reduces the likelihood of non-compliance and inadequate preparation in the elderly [25]. Constipation is another common reason for poor bowel preparation, with increased prevalence in the elderly [26]. A recent systematic review showed the addition of agents such as stimulant laxatives, prokinetic drugs, probiotics and dietary fiber to usual types of oral preparation can improve bowel cleansing quality in patients with constipation, without additional adverse effects [27].

Sedation

Most endoscopic procedures are performed with mild sedation, typically utilizing benzodiazepines, propofol, and opioids such as fentanyl. However, physiological changes in the elderly, use of neurotropic medications, and presence of comorbidities can alter a patient's response to sedatives [28]. Body changes in the geriatric population, including impaired arterial oxygenation, disrupted cardiorespiratory responses to hypoxia and hypercapnia, and central nervous system depression leading to reduced reflexes, including the pharyngeal reflex, need consideration [29,30]. Additionally, increased body fat and impaired renal and hepatic clearance can prolong the effects of certain medications, such as benzodiazepines [31]. The use of neurotropic drugs due to comorbidities can also heighten the risk of oversedation during endoscopy. Consequently, careful selection of sedative dose is required in the elderly to reduce the likelihood of complications such as oversedation and aspiration [17].

Commonly used sedatives for endoscopy include benzodiazepines like midazolam and opioids such as fentanyl. Midazolam is frequently employed, and its co-administration with fentanyl has shown positive results in enhancing procedure tolerance [32]. Propofol is favored for achieving better tolerance during lengthy endoscopic procedures due to its short half-life and rapid onset of action [33]. Despite its established safety and tolerability, the lack of a reversal agent and adverse effects including hypotension elevate the potential risks of propofol, especially in the elderly [34].

Preventive measures should be implemented before endoscopy to mitigate the risks associated with sedation. Assessing renal and hepatic function prior to the procedure is essential. Adequate monitoring devices and basic resuscitative equipment must be readily available, with staff appropriately trained in patient assessment and device usage [35,36]. Given that desaturation is more frequent in the geriatric population during endoscopy, continuous oxygen supplementation via nasal cannula must be available. Training in airway protection and on-site availability of suction are also crucial [37]. Careful selection of the sedative agent is imperative, with lower initial and cumulative doses administered at reduced rates / longer intervals to minimize adverse effects in geriatric patients [38].

Sedation-free endoscopy is an alternative to mitigate the potential risks associated with sedative agents, with studies indicating increased tolerance and satisfaction among elderly patients undergoing such procedures [39]. Administering sedation on an as-needed basis during endoscopy, rather than a fixed dose at the start of the procedure, appears to reduce the prevalence of hypotension and hypoxia while also reducing procedural costs [40]. The use of ultrathin endoscopes, measuring 6 mm in diameter compared to the regular 10- to 11-mm endoscope, can facilitate sedation-free endoscopy by reducing sympathetic system activation and oxygen desaturation, thereby minimizing the risk of complications in the elderly [41].

Upper GI Endoscopy in Elderly Patients: Clinical Considerations

Although upper GI endoscopy can provide crucial information that influences clinical decision-making, there are several considerations. A study by Theocharis et al. highlighted severe comorbidity(-ties) as the main determinant of adverse outcomes in octogenarians with upper GI bleeding [42]. In a cohort of 218 patients undergoing esophagogastroduodenoscopy (EGD), indications included symptoms of GI bleeding (41%), anemia (15%), dyspepsia (31%), dysphagia, weight loss and anorexia (9%), and/or reflux symptoms (3%). Serious disease (cancer, peptic ulcer, reflux esophagitis, and/or erosive gastritis/duodenitis) was identified in 44% of cases [43]. A large study of 3147 elderly patients undergoing EGD by Buri et al. reported that findings affecting medical decisions were present in 49.7% of patients, with factors most commonly associated with abnormal findings being male sex, weight loss, bleeding, and symptoms of gastroesophageal reflux disease [44]. A more recent study of 202 patients aged 75 and over who underwent EGD showed a high diagnostic yield and good safety profile. Common indications included dyspepsia, GI bleeding, reflux, anemia, and

screening/surveillance, with malignancy and/or ulcers being detected in 19.3% [45].

The safety and tolerance of EGD in the elderly have been well-documented. Age alone should not serve as a contraindication for upper GI endoscopy. Compared to bowel preparation, preparation for EGD is simpler, thus making it better tolerated by older patients [46]. Lee et al. found no significant differences in hospitalization duration, need for repeat endoscopy, transfusions, endoscopy complications, or mortality between younger and elderly patients requiring therapeutic upper GI endoscopy [47]. However, the increased prevalence of comorbidities in the geriatric population underscores the importance of thorough pre-procedural examination and cardiopulmonary monitoring during EGD. Caution should be exercised in patients with (known) underlying heart disease, as they run a higher risk of cardiopulmonary complications during the procedure, including arrhythmias and ST wave changes [48]. Markers such as brain natriuretic peptide have been shown to be elevated following upper GI endoscopy, providing further evidence of cardiac strain during EGD. [49]. The presence of pharyngeal abnormalities may impede endoscope progression [50]. Careful attention is necessary to mitigate complications. As previously mentioned, older patients exhibit better tolerance for unsedated endoscopy, further enhancing the procedure's safety [51]. The use of ultrathin endoscopes can reduce sedation requirements, oxygen desaturation, and disturbance of cardiac function [52-53]. Proper head positioning is crucial to prevent potential aspiration events, and caution is warranted in patients with suspected Zenker diverticulum to avoid possible perforation [55].

Colonoscopy in Elderly Patients: Clinical Considerations

The utilization of colonoscopy in the elderly has seen a notable increase in recent decades. Common indications include abdominal pain, rectal bleeding, changes in bowel habits, and a palpable abdominal mass [56]. A significant portion of colonoscopies in the elderly are performed for colorectal cancer (CRC) screening and surveillance [17]. While CRC is one of the most common and deadliest types of cancer globally, screening colonoscopy has demonstrated effectiveness in reducing its incidence [57]. However, the utility of preventive endoscopy beyond a certain age is subject to debate. Despite the higher detection rate of CRC in older patients, the extension in life expectancy and median survival has been shown to be smaller compared to younger patients, potentially due to delayed obvious benefits of screening, deranged functional status and comorbidities [58,59,60]. Complications associated with bowel preparation and the procedure itself are also areas of concern. The American College of Gastroenterology (ACG), the American Cancer Society (ACS) and U.S. Preventive Services Task Force (USPSTF) suggest CRC screening in patients 45 to 75 years of age, and recommend individualized decision-making for screening colonoscopy in patients over 75 years old based on overall patient health, prior screening history, and preferences [61,62,63]. ESGE guidelines recommend screening for CRC in individuals 50 to 75 years of age [64]. Screening for CRC in patients over 85 years of age is discouraged. Elderly patients not previously screened for CRC, those healthy enough to undergo treatment if CRC is detected, and those without substantially limited life expectancy are likely to benefit more from screening colonoscopy [62,63]. Patients should be fully informed of possible procedure risks, with primary care physicians playing a crucial role in counseling, as with any screening test [65]. Special consideration should be given to patient comorbidities, as GI adverse events after colonoscopy appear to be increased in patients with a history of stroke, chronic obstructive pulmonary disease, atrial fibrillation, or congestive heart failure [66]. Alternative screening strategies, such as fecal blood tests and computed tomography colonoscopy, may offer lower complication risks but lower diagnostic accuracy [63,65].

Several studies have investigated the diagnostic yield, safety, and complication rates of colonoscopy in elderly individuals. Karajeh et al. in a study comparing 1000 patients aged over 65 years to 1000 younger patients, found similar completion and adverse event rates between both groups, with a higher diagnostic yield and rates of carcinoma in the older cohort [67]. Another study comparing octogenarians to younger patients demonstrated a high diagnostic yield, increased detection of CRC, and lower sedation requirements in the older group, with similar complication rates in both age groups [68]. These studies provide evidence of the safety of colonoscopy in older patients, although advanced age remains a risk factor for procedure-related adverse events [69]. A meta-analysis by Day et al. reported increased mortality rates and adverse effects during and after colonoscopy in the elderly, with cardiopulmonary events being the most common, potentially exacerbated

by sedation [70]. Serious adverse events, including perforation, GI bleed, and blood transfusions, were more frequent in the geriatric population compared to younger patients in other large retrospective studies [66,69]. Failure of deep cecum intubation and prolonged procedural duration during colonoscopy have been associated with colonic tortuosity, diverticular disease, and poor visualization secondary to inadequate bowel preparation, all of which are more prevalent in geriatric patients [69,71]. Conditions such as prior abdominal surgery and hernias are common in the elderly, and their presence can complicate colonoscopy [72,73]. Experience in performing endoscopy with excellent basic technique along with advanced methods such as water exchange and manual hernia reduction can aid the completion of the procedure in these patients [72,73]. An image of colonoscopy in a patient after manual reduction of a hernia can be seen in Figure 1.

Figure 1. An inguinal hernia protruding into the scrotum in an elderly male patient undergoing investigation for lower GI bleeding. Appropriate manual hernia reduction facilitated endoscope progress with the colonoscope intubating the cecum uneventfully. *Source: Personal records*

Percutaneous endoscopic gastrostomy: Clinical considerations

Upper endoscopy with percutaneous endoscopic gastrostomy (PEG) placement has become increasingly utilized in recent decades for nutritional support in the elderly, as it has demonstrated superiority over parenteral nutrition and surgical procedures for GI access [74]. Mendiratta et al. reported that the primary diagnosis of patients with PEG placement during a 10-year period (median age 80.2 1993-1997 and 80.1 1998-2003, range 65 or older) included cerebrovascular disease (13.7%), aspiration pneumonia (12%), pneumonia (3.11%), malnutrition (1.94%), congestive heart failure (1.78%), and dysphagia (1.36%) [75]. In a prospective cohort study by Callahan et al. (median age 78.9±8.1, range 60-98), common complications included vomiting (30%), diarrhea (26.7%), constipation (22.7%), nausea (20%), and aspiration symptoms (18.7%), while major complications were rare, and no patients died directly due to PEG placement [76].

Significant concerns surround PEG placement, particularly as it does not appear to extend long-term survival in elderly patients [77]. Thirty-day mortality rates after PEG placement seem to rise, primarily attributable to underlying medical comorbidities rather than the procedure itself [78]. A systematic review by Goldberg et al. concluded that there is no evidence to suggest improved long-term survival rates in patients with advanced dementia who undergo PEG placement for dysphagia [79]. PEG feeding also impacts the social aspects of eating, which are vital to elderly individuals with comorbidities such as dementia [80]. Current European Society of Gastroenterology (ESGE) guidelines advise against PEG placement in patients with advanced dementia and those with a life expectancy of less than 30 days [81]. Given the questionable improvement in clinical outcomes and the invasive nature of the procedure, the decision for PEG placement should be individualized, with careful consideration of expected benefits relative to potential risks [81].

Endoscopic Retrograde Cholangiopancreatography (ERCP) in the Elderly: Clinical Considerations

Pancreaticobiliary diseases such as choledocholithiasis and periampullary carcinomas are more prevalent among the elderly population. Endoscopic retrograde cholangiopancreatography (ERCP) emerged as an effective minimal invasion intervention that reduced the need for surgical procedures in the elderly, consequently lowering morbidity and mortality rates [82]. The safety, efficacy, and tolerance of ERCP in older individuals have been extensively demonstrated in the literature. A systematic review by Day et al. indicated that ERCP is generally safe in patients over 65 years of age, with common complications including cholangitis

(1.61%), pancreatitis (1.31%), bleeding (0.77%), perforation (0.38%), cardiopulmonary events (0.37%), and death (0.71%). Nonagenarians were found to be more susceptible to adverse events compared to other age groups [83]. Interestingly, pancreatitis occurred less frequently in the elderly compared to younger patients, with some studies suggesting that a reduction in pancreatic exocrine function with advancing age could offer some protection against post-ERCP pancreatitis [84]. A study reported that biliary obstruction was the leading indication (73.7%) for ERCP in elderly patients, with a low procedural as well as a low sedation-associated complication rate compared to younger individuals [85]. Overall complication rates during or following ERCP, as well as therapeutic success rates, appear to be comparable between the geriatric population and younger patients, thereby emphasizing that late age should not be considered a contraindication for ERCP. [86]. All things considered, ERCP should be preferred over biliary surgery in the elderly, as it reduces the rate of major complications, hospital stay, and risks associated with general anesthesia [87].

Endoscopic Ultrasound (EUS) in the Elderly: Clinical Considerations

Endoscopic ultrasound (EUS) is a procedure characterized by its relatively low risk of adverse events and is commonly utilized for cancer staging, evaluation of pancreaticobiliary diseases, and assessment of subepithelial lesions [15]. However, data regarding the safety and utilization of EUS in the elderly population remain limited. A study by Benson et al. demonstrated that adverse events associated with EUS in the geriatric population were comparable to those observed in younger patients [87]. A retrospective analysis involving 265 patients with a mean age exceeding 80 years revealed that common indications for EUS included evaluation of the pancreas and biliary tree, luminal pathology, subepithelial lesions, and mediastinal pathology. The authors concluded that EUS could safely influence the clinical outcomes of gastrointestinal, pancreatobiliary, and mediastinal diseases in elderly patients [88].

In a retrospective study by Takahashi et al. involving 600 patients who underwent EUS-FNA under midazolam-based sedation, age was not identified as a predisposing factor for adverse events, with the elderly group requiring significantly lower doses of sedation [89]. The simultaneous performance of EUS and ERCP is suggested in elderly patients, with studies showing it is generally safe with similar adverse events in all age groups [90]. The combination of ERCP and EUS is generally well tolerated, useful for diagnosis and therapeutic intervention, and can help reduce costs and avoid unnecessary hospitalizations regardless of patient age and comorbidities [91].

Device-Assisted Enteroscopy (DAE) in the Elderly: Clinical Considerations

Device-assisted enteroscopy (DAE) is a valuable technique that has significantly contributed to the diagnosis and management of various small bowel diseases, particularly in cases of obscure GI bleeding [92]. Three different DAE modalities available in clinical practice are double balloon enteroscopy, single balloon enteroscopy, and spiral enteroscopy [93]. Studies suggest that older age is associated with an increased rate of abnormal findings in DAE [95]. Angioectasias are commonly identified in the geriatric population undergoing DAE, with many cases requiring endoscopic hemostasis. Notably, the procedure duration and adverse effects in older patients do not appear to be significantly elevated compared to younger counterparts [96]. A systematic review of patients who underwent DAE revealed a higher diagnostic and therapeutic yield in elderly patients, along with lower requirements for sedation compared to younger patients [97].

While complications of DAE in the general population include perforation, abdominal pain, bleeding, and pancreatitis [92], its safety and effectiveness in the elderly population are still under investigation. Based on current evidence, DAE emerges as a safe and effective tool that can aid in the diagnosis and treatment of small bowel abnormalities in a population that appears to be more often in need of intervention.

Capsule Endoscopy in the Elderly: Clinical Considerations

Video capsule endoscopy (VCE) is a commonly employed method for investigating small bowel abnormalities, encompassing conditions such as gastrointestinal bleeding, small intestinal polyps, malignancy, malabsorption, and inflammatory bowel disease. While the procedure offers valuable diagnostic insights, potential risks include aspiration, capsule retention, and perforation [98]. Capsule retention due to oesophageal diverticula can be seen in Figure 2. Concerns have also been raised regarding potential interference between small bowel capsules and cardiac implanted devices as

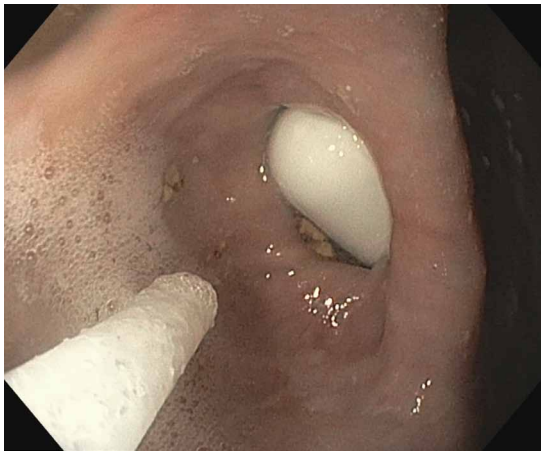

Figure 2. A large diverticulum of the esophagus in an elderly patient resulting in wireless video capsule (PillCam) impaction. A mid-esophageal true esophageal diverticulum is a rare disease. Although it can occur in all ages, they are typically diagnosed in the elderly. *Source: Personal records*

well as insulin pumps [99,100].

A retrospective study of 180 patients who underwent capsule endoscopy revealed that older patients had similar gastric small bowel passing time, increased incidence of angiodysplasias and found the procedure more tiresome compared to younger patients [101]. Additionally, a large cohort study conducted by Gomez et al. demonstrated a higher diagnostic yield (73% compared to 55% in younger patients) and a comparable rate of adverse events in the elderly, with angiodysplasias being the most common finding [102].

Conditions such as hernias, extensive previous abdominal surgery, diverticulosis, fistulas, abdominal radiation or motility disorders such as achalasia require special consideration, due to possible GI stenosis and risk of capsule retention or difficulty swallowing the capsule [103]. Many of these conditions are common in the elderly, and careful history assessment before the procedure is paramount. Patients with suspected GI stenosis, especially those with established Crohn's disease, are advised to undergo pre-testing like use of a patency capsule prior to VCE [104]. Aspiration due to motility disorders can be avoided by endoscopic placement of the capsule into the duodenum [105].

In summary, capsule endoscopy is a safe procedure which can be used effectively to investigate small bowel disease in the elderly. Careful history taking can help determine which patients will require other diagnostic tests to avoid possible complications. Clinical considerations needed before, during and after endoscopic procedures in the elderly, are summarized in Table 1.

CONCLUSIONS

In conclusion, diagnostic and therapeutic endoscopy can be safely conducted in elderly patients, provided that appropriate pre-procedural assessment and preparations are undertaken. This includes solid indications, meticulous attention to comorbidities, modification of

Table 1. *Clinical considerations needed before, during and after endoscopic procedures in the elderly.*

Before endoscopy	During endoscopy	After endoscopy
Risk-benefit assessment	Monitoring vital signs, oxygen supplementation	Monitoring for oversedation
Assessment of Cardiopulmonary status, renal and liver function	Possible electrical interference by devices such as pacemakers and defibrillators	Possible Cardiopulmonary events
Ability to consent, obtain consensus from family members if necessary	Sedation dosage, consider sedation- free endoscopy	Post endoscopy bleeding, possible need for transfusions
Drug modifications (e.g. antidiabetics, antiplatelets)	Use of ultra-thin endoscopes	Other complications (e.g. perforation, pancreatitis, cholangitis)
Careful choice of bowel preparation regimen	Anatomic abnormalities complicating endoscopy (e.g. hernias, diverticulae, GI stenosis in Crohn disease)	
Proper head placement to avoid aspiration		

drug doses as necessary and thorough explanation of the procedure and potential complications. Utilizing polyethylene glycol solutions and split dosage regimens can enhance bowel preparation in older individuals. Additionally, sedation-free endoscopy may be considered due to improved procedure tolerance in the geriatric population.

Close monitoring of vital signs is paramount during all endoscopic procedures. Screening for colorectal cancer after the age of 75 should be individualized, taking into consideration factors such as prior screening history and life expectancy. Ultimately, the decision to undergo screening should be based on an assessment of expected risk and its contribution to medical decision-making. By adopting these principles, healthcare providers can ensure the safe and effective delivery of endoscopic care to elderly patients.

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T-cell immunodeficiencies caused by infectious diseases

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Abstract

Certain infectious diseases may lead to the development of secondary T-cell immunodeficiency which is described as a transient or persistent impairment of T-cell function and/ or a decrease of T-cell numbers. HIV infection stands as a quintessential example demonstrating the impact of an infectious disease on T-cell function, leading to the development of acquired immunodeficiency syndrome (AIDS). Viral infections, including measles, have been associated with a transient period of immunosuppression, while COVID-19 induces a profound dysregulation of T-cell responses. Parasitic infections, such as malaria and leishmaniasis manipulate the host's immune system and impair T-cell function by inducing T-cell anergy and exhaustion. Bacterial infections, such as tuberculosis may also lead to the disruption of cellular immune responses. This article examines the range of mechanisms employed by infectious diseases, which contribute to the occurrence of T-cell immunodeficiency.

Key words: *T-cell immunodeficiency; HIV; COVID-19; measles; malaria; leishmaniasis; tuberculosis*

INTRODUCTION

T-cell immunodeficiency can emerge either as a primary disorder or as a secondary condition resulting from various underlying causes. Primary immunodeficiency syndromes are a heterogenous group of inborn errors of immunity that typically arise from single gene disorders or other maturational abnormalities [1]. In contrast, common causes of secondary immunodeficiencies include infection, medications, malignancy, malnutrition, age extremes and other environmental factors [2]. Secondary immunodeficiency might be defined as a transient or persistent acquired decline of immune cell counts and /or function [3].

T lymphocytes originate from bone marrow progenitors, which then migrate to thymus for maturation, selection and subsequent release into the peripheral circulation. Two major T-cell subsets are defined by the additional expression of either CD4 or CD8 molecules on the cell surface [4]. CD8+ cytotoxic T cells are primarily involved in the destruction of cells infected by foreign agents, such as viruses, and tumor cells expressing appropriate antigens. CD4+ T cells are important for establishing and maximizing the immune response. Through differentiation into T helper cell subsets, they regulate the type of immune response that develops and directs various immune cells, including B cells, macrophages and CD8+ T cells in executing their designated functions [5]. However, some pathogens have evolved mechanisms to interfere with these effector immune responses, manipulate or exhaust the host's immune system, thereby inducing secondary immunodeficiency [6].

Secondary T-cell immunodeficiency is the primary characteristic of HIV infection [1]. Also, viral infections, including measles, have been associated with transient periods of immunosuppression [7]. Moreover, in several chronic infections persistent antigen stimulation induces decreased T cell effector functions, a condition known as T-cell exhaustion [8]. Various studies suggest that this phenomenon can be extended to protozoan diseases as

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well [9]. Another strategy employed by parasitic infections to downregulate T-cell function is the induction of T-cell anergy [10]. The objective of this review is to discuss the diverse immunologic mechanisms employed by infectious diseases leading to the emergence of T cell immunodeficiencies (Table 1).

Table 1. *T-cell dysregulation associated with infectious diseases.*

Infectious Disease	Affected cells	Immune dysregulation phenotype	Mechanism implicated	Duration of immunosuppression
HIV/AIDS	CD4+ T cells	(1) CD4+ T cell depletion (2) Chronic immune activation	Extrinsic and intrisic apoptosis pathway Activation-induced cell death	Long-term
Covid-19	T cells and APCs	(1) Restricted antigen presentation (2) Reduction of lymphocyte counts (3) T-cell exhaustion	Downregulation of MHC molecules Cytokine storm induction Apoptosis Upregulation of inhibitory receptors	Temporary
Measles	T cells and B cells	(1) Reduction of lymphocyte counts (2) Immune amnesia	Apoptosis and interference with cell cycle phases Altered lymphocyte trafficking Memory cell depletion	Temporary
Leishmaniasis	T cells	(1) T-cell anergy (2) T-cell exhaustion	Lack of co-stimulatory molecules Expression of immunomodulatory molecules Upregulation of inhibitory receptors	Temporary
Malaria	T cells and Dendritic Cells	(1) Impaired antigen presentation (2) T-cell exhaustion	Downregulation of MHC molecules Upregulation of inhibitory receptors	Temporary
Tuberculosis	T cells and APCs	(1) Impaired antigen presentation (2) T-cell exhaustion (3) Reduction of lymphocyte counts	Interference with DC maturation Upregulation of inhibitory receptors Apoptosis Granuloma formation	Temporary

HIV / AIDS

HIV infection has been a global public health issue for over 4 decades and has been associated with more than 40 million deaths [11]. HIV is a single-stranded, enveloped RNA retrovirus from the genus Lentivirus within the family of Retroviridae and is classified into two types, HIV-1 and HIV-2, both of which cause disease in humans. The HIV genome consists of 3 structural genes (gag, pol and env) and six regulatory genes (tat, rev, nef, vif, vpr, and vpu) [12]. HIV infection causes a progressive, multifactorial impairment of the immune system eventually leading to the acquired immunodeficiency syndrome (AIDS) [13]. Systemic chronic immune activation and CD4+ T cell depletion are the hallmarks that characterize the progression of the infection towards immunodeficiency [14].

The infection begins with Env protein, composed of gp120 and gp41 subunits, binding to CD4 molecule and chemokine receptors CCR5 or CXCR4 on target cells [15]. During acute infection CD4+ T cells are severely depleted, especially in gut-associated lymphoid tissue (GALT) which harbors the majority of T lymphocytes in the body [16,17]. There are several mechanisms by which HIV leads infected cells into cell death. HIV induces syncytia formation by the fusion of infected cells expressing Env with the uninfected target expressing a suitable coreceptor (CD4 or CCR5). Syncytia have a short life span and are condemned to die by apoptosis due to genomic instability [18]. In addition, other direct cytopathic effects of HIV on infected cells compromise cell viability. Specific HIV proteins can trigger extrinsic and intrinsic apoptosis pathways. Tat (Trans Activating Factor) is a regulatory HIV protein that has been shown to upregulate CD95 and FasL levels thus enhancing susceptibility to Fas-mediated killing [19]. HIV protease can inactivate anti-apoptotic Bcl-2, while simultaneously activating pro-apoptotic procaspase 8 leading cells to mitochondrial-dependent pathway of apoptosis [20,21]. Furthermore, HIV induces cell death in uninfected cells either by HIV proteins released from infected cells acting on neighboring uninfected cells or by activation-induced cell death [22].

HIV infection also induces chronic immune activation. Viral gene products (Nef, Tat, Vpr, Vpu) and inflammatory cytokines contribute to immune activation by stimulating various immune cells such as monocytes, macrophages and dendritic cells. This hyperimmune activation is characterized by an increased T-cell turnover, non-specific T-cell activation and proliferation, polyclonal activation of B cells and elevated proinflammatory cytokines [23]. Although this leads to increased cell counts, the activated CD4+ T cells have a short life span and are rapidly depleted due to activation-induced cell death or apoptosis. Also, the massive production of proinflammatory cytokines leads to clonal deletion and gradual loss of peripheral CD4+ T cells over time [23].

Without treatment CD4+ T cell counts and immune responses progressively decrease rendering the host susceptible to infections with opportunistic pathogens. Peripheral CD4+ T cell counts below 200 cells/mL mark the onset of acquired immunodeficiency syndrome (AIDS) and patients can present with any number of infections that define AIDS, such as *Pneumocystis jirovecii* pneumonia, histoplasmosis, toxoplasmosis and coccidioidomycosis [7].

CORONAVIRUS DISEASE 2019

Coronavirus disease (COVID-19) is a disease caused by the severe acute respiratory syndrome coronavirus 2 (*SARS-CoV-2*), which emerged as a pandemic since it was first reported in late 2019. Clinical manifestations of the infection range from asymptomatic forms to severe forms with life-threatening pathologies such as acute respiratory distress syndrome (ARDS) [24]. It is now well established that SARS-CoV-2 infection can profoundly affect the functionality of the immune system, leading to dysregulation and immune cell exhaustion [25]. Similar to other viral infections, T cell-mediated immunity plays a vital role in recognizing and controlling SARS-CoV-2 infection. However, T cell dysregulation has been associated with the development of severe disease as well [26,27].

SARS-CoV-2 is able to restrict antigen presentation through downregulation of major histocompatibility complex (MHC) class I and II molecules and consequently lead to the inhibition of T cell-mediated immune responses [28,29]. Also, there is evidence that the virus can suppress and delay the production of type I IFN by expressing factors such as ORF6 and non-structural proteins (nsp) 1 and nsp6 [30,31]. As IFNs promote the survival and effector functions of T cells, impaired T-cell responses can result from deficient IFN production. However, further research is warranted to confirm this possibility [32].

Severe COVID-19 is associated with impaired T-cell responses that manifest as lymphopenia and functional exhaustion of CD4+ and CD8+ T cells. SARS-CoV-2 infection predominantly impacts T lymphocytes, especially

CD4+ and CD8+ T cells, leading to a reduction in absolute counts, particularly evident in severely ill patients [33,34]. Studies demonstrated a negative correlation between the viral RNA and CD4+ and CD8+ T cells, suggesting that lymphopenia influenced by SARS-CoV-2 RNA, was closely related to disease severity [35]. Detectable serum SARS-CoV-2 RNA was associated with elevated IL-6 concentration as well [36]. Also, increased concentrations of cytokines, including IL-2R, IL-6, TNF-α and IL-10, were detected in the majority of severe cases, suggesting that cytokine storms might be associated with disease severity [33]. Hence, several mechanisms may act together and overlap in some cases to cause lymphopenia. Direct effects of SARS-CoV-2 on T cells can induce apoptosis. Additionally, lymphopenia may be driven by inflammatory responses like cytokine storm-induced apoptosis and tissue redistribution of lymphocytes [37]. Moreover, T-cell exhaustion may contribute to the observed lymphopenia [34].

T-cell exhaustion is a gradual process of cell function loss, at first detected as a reduced production of IL-2 and proliferative response of CD8+ T cells, followed by loss of ability to produce TNF-α and diminished cytotoxic effect. Later on, complete loss of ability to produce IFN-γ and some chemokines is observed. Eventually this process leads to the loss of effector function, depleted proliferative capacity, suppression of cytotoxic T-cell response and cell death. Along with CD8+ T cells the CD4+ T cells also undergo loss of effector function [38]. Exhausted T cells are characterized by an increased and persistent expression of inhibitory receptors and an altered transcriptional profile [8]. Studies on COVID-19 patients demonstrated an increased expression of inhibitory receptors, including immunoglobulin mucin-3 (TIM-3) and programmed cell death protein-1 (PD-1) on T cells that are associated with functional exhaustion of CD8+ T cells [39]. Also CD8+ T cells which had upregulated NKG2A protein, exhibited an exhausted phenotype [40]. Further research indicated that CD4+ and CD8+ T cells are exhausted in patients who have reduced expression of IFN-γ and IL-21 [39].

Measles

Measles is a highly contagious, potentially fatal but vaccine-preventable disease caused by measles virus. The infection begins in the respiratory tract and systemically spreads to infect multiple organs. Clinical presentation varies, ranging from mild symptoms such as fever, rash and conjunctivitis, to more severe manifestations like pneumonia and encephalitis [41]. In addition, measles was the first infectious disease recognized to increase susceptibility to other infections. A transient period of immunosuppression associated with the infection was initially documented in 1908, when a decrease in tuberculin skin reactivity in measles patients was observed [42]. Although the exact mechanisms remain unclear, lymphopenia, inhibition of lymphocyte proliferation and immune amnesia contribute to the development of immunosuppression.

During measles infection, lymphopenia occurs at the onset of the rash in the majority of measles cases with a decrease in cell numbers of CD4+ T cells, CD8+ T cells, B cells and other cell types. The expression of apoptosis-associated molecules, such as CD95 (Fas) and TNF-related apoptosis-inducing ligand-receptor (TRAIL-R), was upregulated on the cell surface of surviving lymphocytes suggesting that lymphopenia was related to apoptosis [43]. Other types of cell death as well as alterations in lymphocyte trafficking may contribute to lymphopenia [44]. However, as the rash resolves, lymphocyte counts return to normal. In addition, measles virus induces suppression of T-cell proliferation through direct inhibitory signaling to T cells by the viral glycoprotein complex of hemagglutinin (H) and fusion (F) in the membrane of virions or infected cells [45,46]. This inhibitory signal prevents the entry of T cells in the S phase leading to cell cycle retardation and accumulation of cells in G0/G1 phase [47]. Lastly, measles infection induces immune amnesia by infecting and eliminating pre-existing memory T cells as well as B cells that express high levels of CD150. As a result, memory cell repertoires are depleted of many specificities leading to increased susceptibility to infections that are unrelated to measles [48].

Leishmaniasis

Leishmaniasis is a vector-borne tropical disease caused by a diverse group of protozoans of the genus *Leishmania*. Leishmaniasis is transmitted by female phlebotomine sandflies causing a wide range of clinical syndromes. There are three main clinical manifestations in humans. Cutaneous leishmaniasis (CL) is the least severe form of the disease and is caused by several species such as *Leishmania major, Leishmania tropica, Leishmania mexicana* and *Leishmania amazonensis.* Mucocutaneous leishmaniasis (MCL) is caused by *L. braziliensis.* Visceral leishmaniasis (VL) is the most severe form of the disease that results from the infection with *Leishmania donovani*

and *Leishmania infantum* strains [49].

Infection with *L. amazonensis* has been associated with the induction of T-cell anergy to both related and unrelated antigens [50]. T-cell anergy is described as a state of non-responsiveness at the time of T cell stimulation through T cell receptor either due to the absence of costimulatory signals or the expression of immunomodulatory molecules by antigen presenting cells (APCs) [10]. A proposed mechanism for the induction of T cell anergy involves the production of TGF-β by macrophages [50]. Also, in chronic infection persistent antigen presentation promotes T cell exhaustion. During *L. mexicana* infection dendritic cells (DCs) produce high amounts of TNF which compromises the proliferation and functionality of T cells [51]. Additionally, chronic VL leads to T cell exhaustion through the upregulation of several inhibitory receptors such as PD-1 and CTLA-4 [52,53]. The expression of endogenous mediators, including hypoxia inducible factor 1-α (HIF-1α) and adenosine may also contribute to T cell exhaustion [54]. Lastly, several studies demonstrate that T cell apoptosis which occurs during leishmaniasis, may impact the mechanisms of T cell memory formation and compromise immunity during chronic infection [10].

Malaria

Malaria is an endemic vector-borne parasitic disease caused by protozoan parasites of the genus *Plasmodium* in tropical and subtropical regions worldwide. Among over 200 species of *Plasmodium*, *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* and *Plasmodium knowlesi* are known to cause disease in humans [55]. Malaria is associated with public-health problems resulting from impairment of immune responses [56]. There is evidence that malaria can induce immunosuppression in infected individuals, resulting in increased susceptibility to secondary infections such as non-typhoidal *Salmonella* [57], HBV [58] and herpes zoster virus (HSV) [59].

The suppression of immune function seen in malaria infection could be attributed to a parasite induced dysfunction of the DCs [60]. Interactions between antigen-presenting DCs and T cells are essential for the induction of an immune response. A parasite-induced failure in DCs function affects the generation of T-helper cell responses. These T cells fail to help B cell responses, reducing the production of antibodies that are necessary to control malaria infection [61]. Various studies have shown that DC phenotype is altered during malaria infection resulting in impaired ability to upregulate the MHC molecule HLA-DR and costimulatory molecules CD86 [62–65]. This altered phenotype has a reduced phagocytic capacity which impairs its ability to process antigens and prime T-cell responses. Another study demonstrated that the reduced effector function of CD4+ T cells during malaria is due to an inability to form a stable, long-lasting connection between T cells and DCs [66].

Furthermore, T-cell exhaustion plays a role in the impairment of T-cell function during malaria infection. Prolonged infection results in dysfunctional parasite specific CD4+ T cells that express exhaustion markers. The upregulation of PD-1 and lymphocyte-activation gene-3 (LAG-3) inhibits T-cell function and affects the ability of CD4+ T cells to produce cytokines [67]. Specifically, PD-1 mediates a reduction in the capacity of parasite-specific CD4+ T cells to proliferate and secrete IFN-γ and TNF-α [68]. Also, a study demonstrated that a dual blockade of PD-1 and LAG-3 improves CD4+ follicular T helper cell numbers and provided evidence that supported the involvement of these inhibitory molecules in T-cell exhaustion during malaria infection [69].

Tuberculosis

Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis*. Individuals with certain conditions, including HIV or AIDS are predisposed to an increased susceptibility to the disease. Worldwide, TB ranks as the second most deadly infectious disease, following COVID-19 with estimated mortalities of 1.3 million in 2022 [70]. The adaptive immune response mediated by T cells is critical for control of *M. tuberculosis* infection [71]. However, *M. tuberculosis* has employed multiple strategies in order to evade the host's immune response and undermine T-cell function and survival.

M. tuberculosis delays T-cell priming by impairing DC maturation and interfering with efficient antigen presentation with a variety of mechanisms [72]. However, even with the presence of an effective primary immune response in many cases the lack of sterilizing immunity poses a great challenge [73]. Persistent antigen stimulation leads effector T cells to functional exhaustion by upregulating the expression of inhibitory receptors, such as PD-1 and TIM-3 [74,75]. In addition, one of the main features of the immune response to *M. tuberculosis* is the formation of an organized structure called granuloma [76]. Although granulomas are important in host protection, they also facilitate persistent infection

by establishing an immunosuppressive environment in which IL-10 impairs Th 1 cell response and lysis of infected macrophages by CD8+ T cells [73]. Moreover, within the granuloma transforming growth factor-β restricts CD4+ T-cell function and survival [77]. It is also worth noting that severe TB is associated with a decrease in both CD4+ and CD8+ T-cell numbers [78,79]. The underlying mechanism of lymphopenia remains unclear. Despite this, various mechanisms including inhibition of lymphocyte proliferation by macrophages, *M. tuberculosis*-induced apoptosis of T cells and *M. tuberculosis*-mediated bone marrow hematopoietic dysfunction may contribute to the occurrence of lymphopenia. Lymphopenia highlights the impairment of immune function and may lead TB patients to general immunosuppression [80].

CONCLUSION

It is well established that viruses, bacteria and parasites have evolved multiple strategies in order to evade and interfere with the host's immune system. The interaction between the pathogen and the host's immune system often results in a profound dysregulation of immune responses that favor the pathogen's survival and have serious consequences for the host. Within the context of a severe primary infectious disease, T-cell defects involve multiple underlying immunological mechanisms, and it can be therefore difficult to demonstrate and quantify the degree of cellular immunodeficiency. Also, distinguishing patients with a secondary T-cell immunodeficiency from patients with an underlying primary T-cell defect is challenging. In clinical settings, maintaining a heightened clinical suspicion regarding secondary T-cell immunodeficiencies is crucial when encountering patients presenting with recurrent infections and abnormal immunologic assessments.

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The current evidence of abbreviated dual antiplatelet therapy duration

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Abstract

The optimal duration of Dual Antiplatelet Therapy (DAPT) following Percutaneous Coronary Intervention (PCI) remains a topic of significant debate and investigation. This review examines the rationale for DAPT shortening duration in specific populations. We discuss the challenges associated with balancing ischemic and bleeding risks in patients undergoing PCI, highlighting the importance of tailored treatment approaches based on individualized risk profiles. Furthermore, we delve into the evidence supporting abbreviated DAPT in high-risk patient cohorts, including those with increased bleeding risk, comorbid malignancies, diabetes mellitus, and complex coronary artery disease. Emerging data suggest that abbreviated DAPT regimens offer comparable efficacy in preventing thrombotic events while reducing bleeding complications. In conclusion, the ongoing evolution of DAPT underscores the need for evidence-based, patient-centered approaches to optimize outcomes for patients undergoing PCI.

Key words: *DAPT; dual antiplatelet therapy; PCI*

INTRODUCTION

The administration of antiplatelet treatment is a crucial element in managing patients undergoing Percutaneous Coronary Intervention (PCI) for Chronic Coronary Syndromes (CCS) or Acute Coronary Syndromes (ACS), aimed at preventing stent thrombosis, restenosis and adverse events, like myocardial infarction or ischemic stroke. Optimal therapeutic strategies, including the selection, combination, timing, and duration of therapy, require meticulous evaluation of various patient and procedural factors. Treatment decisions should carefully balance the benefits of antithrombotic therapy against the potential for severe and/or life-threatening bleeding [1].

According to the current guidelines of the European Society of Cardiology (ESC), DAPT is recommended as the standard for 6 months for patients with CCS and for 12 months following an ACS. However, this duration may be extended for high-ischemic risk patients and shortened for those at high risk of bleeding [2-3].

In recent years, the optimal duration of DAPT has provoked significant controversy and debate. Numerous

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Randomized Control Trials (RCTs) and meta-analyses have suggested alternative treatment approaches for patients with CCS or ACS. These proposals entail shortening the duration of DAPT to one-three or three–six months after PCI and transitioning to P2Y12 monotherapy as a form of de-escalation [4].

This review intends to provide a comprehensive overview of the existing evidence regarding abbreviated DAPT duration with a focus on the management of patients with specific comorbidities, anatomical and technical issues during PCI.

Why is DAPT Shortening important?

The ongoing advancements in stent technologies, coupled with the introduction of novel antiplatelet agents, and the broadening of indications to encompass an aging and medically complex population with anatomically intricate coronary artery disease (CAD), present formidable challenges in the management of patients undergoing percutaneous coronary interventions even in 2024 [5].

The delicate balance between high-risk factors for bleeding and thrombosis comprises a daily clinical dilemma. Both the thrombotic and the hemorrhagic risk are higher during the first months after PCI, thereafter they decrease and remain relatively stable over time [6]. Moreover, major bleeding impacts on mortality comparably to or exceeding that of major ischemic events. On the other hand, minor bleeding incidents may lead to unplanned cessation of antiplatelet therapy, potentially resulting in an increased occurrence of ischemic events. Hence, patients at high bleeding risk may benefit from modifying either the components or the duration of DAPT. Among the strategies investigated to mitigate bleeding events, shortening the duration of DAPT has been the most extensively studied approach [7].

Another issue that may be addressed by the shortening of DAPT duration is patient compliance to long-term medication regimens. Non-adherence rates for post-myocardial infarction patients are recorded to range from 13% to 60% for prescribed, evidence-based medications [8]. Noncompliance to DAPT medication as a secondary prevention strategy, after percutaneous coronary intervention has been reported to be the strongest independent predictor for stent thrombosis [9], as a quadruplicate incidence of a recurrent event (11.0% vs 2.8%; *P*=0.044) and duplicate hospitalization rates (21.2% vs 9.9%; *P*=0.04) have been demonstrated. Premature cessation of dual antiplatelet therapy might be not only influenced by the drugs adverse effects, such as bleeding, dyspnea, or gastrointestinal symptoms but also by psychological factors such as depression and anxiety, which could be potentially linked to the complications associated with coronary artery disease [10-11].

Lastly, abbreviated DAPT may reduce the duration of polypharmacy, a very common condition for people suffering from CAD, because apart from the multiple medications that are necessary for secondary prevention such as antiplatelet, antihypertensive, hypolipidemic drugs etc., many of these patients suffer concomitantly from additional comorbid conditions. Except for the extensive costs in all healthcare systems, polypharmacy elevates the likelihood of inappropriate medication utilization and is associated with higher hospitalization and mortality rates [12].

Current evidence supporting the abbreviation of DAPT

A. General Population

The development of newer generations of drug-eluting stents (DES) with refined antiproliferative agents and reduced strut thickness has resulted in decreased stent thrombosis rates while maintaining low restenosis rates. Additionally, more potent P2Y12 inhibitors such as prasugrel and ticagrelor have emerged as viable options for single antiplatelet therapy. Furthermore, the use of intravascular imaging has contributed to advancements in intervention techniques [13].

Consequently, several randomized controlled trials have been conducted to assess the safety of reducing the duration of dual antiplatelet therapy compared to standard duration. Recently, various meta-analyses of the largest published randomized controlled trials comparing abbreviated (one–three months) DAPT with standard-term (six–twelve months) DAPT in patients undergoing percutaneous coronary intervention with DES for both acute and chronic coronary syndromes have been conducted. They all concluded that one–three-month DAPT reduces major bleeding without increasing ischemic events compared to longer DAPT durations, thus providing a better risk-benefit profile. However, further research is necessary to determine the optimal single antiplatelet agent after one-three months of DAPT [14-16]. These results came to be verified by the SHARE trial, a multicenter RCT that was last month published and investigated a composite of major bleeding and major adverse cardiac and cerebrovascular

events between three and twelve months DAPT after the index PCI.

Correct identification and risk stratification of patients with characteristics of high ischemic or high bleeding risk are crucial, as this subgroup stands to benefit the most from tailored DAPT (Table 1).

Table 1. *Largest* RCTs [45-59] concerning DAPT Shortening published in the last 5 years.*

Name	Publication Year	No. of Patients	Objective	Primary Endpoint	Primary Endpoint met?	Follow up
General Population						
ONE MONTH DAPT	2021	3020	1-month DAPT followed by aspirin monotherapy after PF-DCS implantation vs 6 to 12 months of DAPT after BP-DES implantation	Cardiac death, nonfatal myocardial infarction, target vessel revascularization, stroke, major bleeding	yes	1 Year
SMART CHOICE	2019	2993	3-month DAPT vs 12 months DAPT in patients undergoing PCI	MACEs and cerebrovascular events at 12 months	Yes	1 Year
TWILIGHT	2019	9006	3-month Ticagrelor based DAPT vs 12-month in high ischemic or high bleeding risk patients	BARC type 2, 3, or 5 bleeding	yes	1 Year
HOST- IDEA	2023	2013	3- to 6-month or 12-month DAPT after PCI	NACEs at 12 months	Yes	1 Year
SHARE	2024	1452	3 months or 12 months DAPT post PCI	Major bleeding and MACEs between 3 and 12 months after the index PCI	Yes	1 Year
High Ischemic Risk Patients						
STOP DAPT-2 ACS	2022	4169	Clopidogrel monotherapy after 1-2 months of DAPT vs 12-month DAPT in patients with ACS	cardiovascular or bleeding events at 12 months	No	1 Year
IDEAL - LM	2022	818	long-term clinical outcomes after implantation of a BP-PtCr-EES followed by 4-months DAPT compared to a DP-CoCr-EES followed by 12 months DAPT in patients undergoing PCI of unprotected LMCA	MACEs: all-cause death, myocardial infarction, or ischemia-driven target vessel revascularization at 2 years.	Yes	2 Years
TWILLIGHT-ACS	2020	7119	3-month Ticagrelor based DAPT vs 12-month DAPT in NSTE-ACS patients undergoing PCI with DES	BARC type 2, 3, or 5 bleeding	Yes	1 Year
TICO	2020	3056	ticagrelor monotherapy after 3-month DAPT vs ticagrelor-based 12-month DAPT in patients with ACS treated with DES	major bleeding, MACEs and cerebrovascular events	yes	1 Year
REDUCE	2019	1496	3-month vs 12-month DAPT in ACS patients undergoing new-generation DES implantation	all-cause mortality, myocardial infarction, stent thrombosis, stroke, target vessel revascularization	yes	1 Year

Table 1. *Largest* RCTs [45-59] concerning DAPT Shortening published in the last 5 years (continued).*

Name	Publication Year	No. of Patients	Objective	Primary Endpoint	Primary Endpoint met?	Follow up
High Bleeding Risk						
MASTER DAPT	2021	4434	1-month vs 3-month DAPT post PCI with a BP-SES at HBR patients	NACEs (death from any cause, myocardial infarction, stroke, or major bleeding)	yes	11 months
TWILLIGHT-HBR	2021	1064	3-month Ticagrelor based DAPT vs 12-month DAPT post PCI in HBR	BARC type 2, 3, or 5 bleeding	Yes	1 Year
XIENCE Short DAPT	2021	3652	1-month vs 6-month and 3-month vs 12-month DAPT on HBR patients after PCI with CoCr-EES	all-cause death or myocardial infarction	Yes	1 Year
GLASSY	2019	7585	1-month DAPT followed by 23-month ticagrelor monotherapy or conventional 12-month DAPT followed by 12-month aspirin in HBR patients	all-cause death, nonfatal MI, nonfatal stroke, or urgent target vessel revascularization and superior in preventing BARC 3 or 5 bleeding at 2 years	No	2 years

DES: drug-eluting stent, BP-DES: biodegradable-polymer, BP-SES: biodegradable-polymer sirolimus eluting stent, PF-DCS: polymer-free drug-coated stent, NACE: net adverse clinical events, BARC: Bleeding Academic Research Consortium, MACE: major adverse cardiovascular events, BP-PtCr-EES: biodegradable polymer platinum-chromium everolimus-eluting stent, DP-CoCr-EES: durable polymer cobalt-chromium everolimus-eluting stent, LMCA: left main coronary artery, SCAD: Stable Coronary Artery Disease, NSTE-ACS: Non-ST elevation Acute Myocardial Infarction, HBR: High Bleeding Risk, PCI: Percutaneous Coronary Intervention, DAPT: Dual Antiplatelet Therapy, CoCr-EES: cobalt-chromium everolimus-eluting stents

*Including more than 500 patients

B. Shortening DAPT in patients at elevated risk of bleeding

In routine clinical practice up to 40% of individuals undergoing percutaneous coronary intervention are classified as patients at high bleeding risk (HBR). However, this intricate population is frequently excluded or inadequately represented in trials addressing the management of patients post-PCI [17]. Recently, the Academic Research Consortium for High Bleeding Risk (ARC- HBR) developed a consensus definition of patients at high bleeding risk based on the published evidence [18]. Factors associated with an elevated hemorrhagic risk include age over 75 years, chronic kidney disease, use of oral anticoagulation, anemia, moderate thrombocytopenia, liver cirrhosis with portal hypertension, active malignancy, chronic bleeding diathesis, spontaneous bleeding requiring hospitalization or transfusion, intracranial bleeding, long-term use of oral NSAIDs or steroids, recent major surgery, or surgery under DAPT (Figure 1).

In patients presenting such characteristics, given the ability of new generation stents to heal rapidly, extending DAPT over one-three months might be unnecessary [19]. In the last years, numerous registries have investigated this scenario. Recently, Costa et al., in a recent meta-analysis of eleven trials and 9006 patients compared the outcomes of very short (one month) or short (three months) with standard (≥six months) DAPT duration in HBR patients. Abbreviated DAPT reduced major bleeding (RR: 0.80, 95% CI: 0.64-0.99, I2 = 0%) and cardiovascular mortality (RR: 0.79, 95% CI: 0.65-0.95, I2 = 0%) compared with standard DAPT. Moreover, no difference in all-cause mortality, major adverse cardiovascular events, myocardial infarction, or stent thrombosis was observed [20].

These findings reinforce the safety and efficacy of abbreviated DAPT in patients classified as high bleeding risk, advocating its inclusion as a potential treatment strategy.

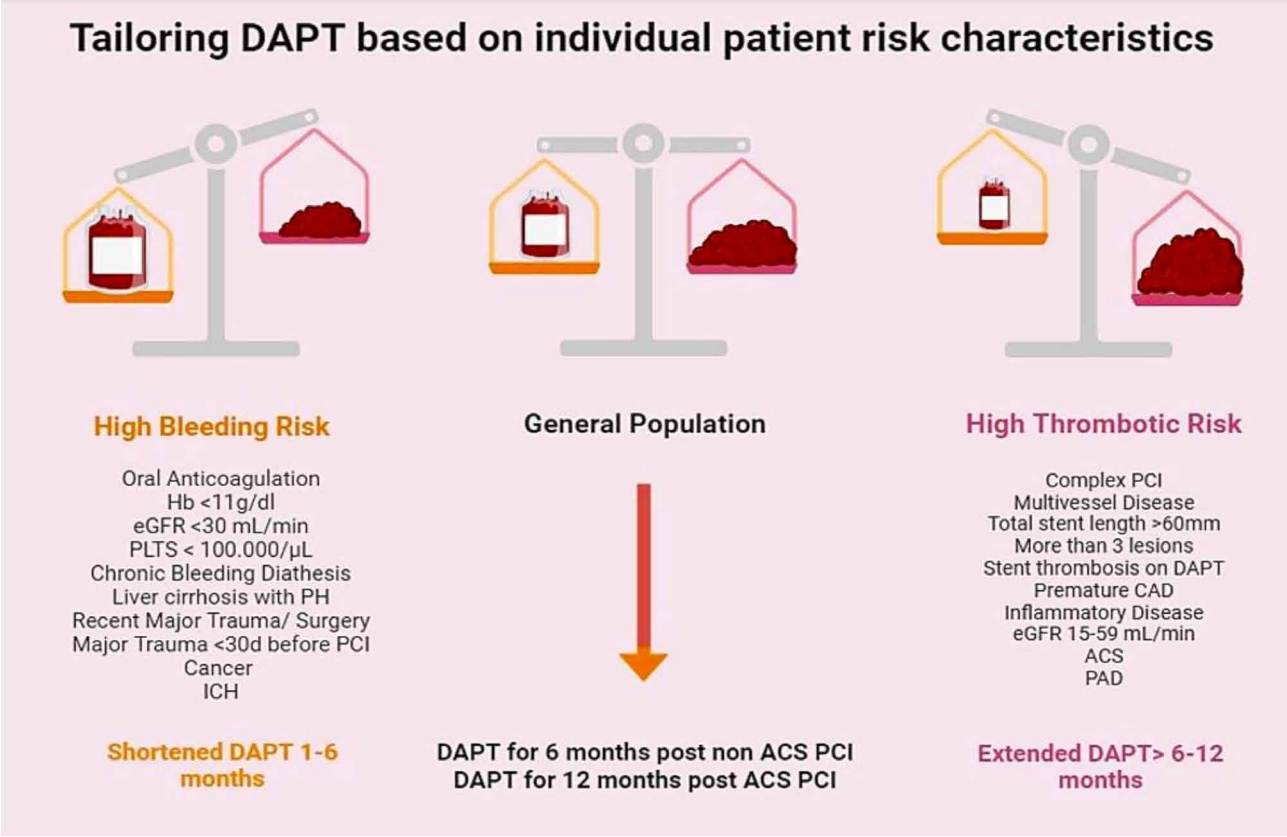

Figure 1. High Bleeding/ Ischemic Patient Characteristics and ESC Guidelines recommendations on DAPT.
Hb: Hemoglobin, GFR: Glomerular Filtration Rate, PLTs: Platelets, PH: Portal Hypertension, ICH: Intracranial Hemorrhage, PCI: Percutaneous Coronary Intervention, DAPT: Dual Antiplatelet Therapy, ACS: Acute Coronary Syndrome, SCAD: Stable Coronary Artery Disease, PAD: Peripheral Arterial Disease, CAD: Coronary Artery Disease.

C. Shortening DAPT in patients at elevated risk of ischemia

The recent ESC guidelines recommend a definition for patients at high or moderate thrombotic risk to aid in stratifying thrombotic risk and guiding intensified antithrombotic treatment following the standard duration of dual antiplatelet therapy. These categories encompass individuals with complex coronary artery disease, diabetes mellitus (DM), recurrent myocardial infarction, premature or accelerated CAD, concomitant peripheral arterial disease, or systemic inflammatory disease [17-18]. In addition, technical aspects such as the implantation of at least three stents, a total stent length of 60mm or more, complex revascularization, or stent thrombosis during antiplatelet therapy are considered in these recommendations (Figure 1).

In the following paragraphs, we will also discuss the management of specific populations of individuals who are at an elevated risk of ischemia.

Tailoring DAPT for Specific Patient Population

A. DAPT after PCI in patients with Diabetes Mellitus

Worldwide, greater than 30% of individuals with diabetes concurrently experience cardiovascular disease, which is responsible for approximately half of all deaths [21]. This phenomenon may be potentially attributed to the presence of multifocal coronary artery lesions, which is often observed in these patients, as well as their increased susceptibility to stent restenosis [22]. Furthermore, resistance to antiplatelet therapy has been observed in these patients [23].

According to current guidelines, DAPT post-percutaneous coronary intervention in diabetic patients is recommended for a duration ranging from three to twelve months. However, the duration may be extended up to 30 months based on the physician's clinical judgment [2-3].

A systematic review and meta-analysis by Gargiulo et al., which included 11,473 participants, investigated the clinical outcomes of short-term (≤six months) versus

long-term (twelve months) dual antiplatelet therapy following PCI in patients with and without diabetes. The findings indicated that although diabetes was identified as an independent predictor of major adverse cardiac events (MACE) (HR 2.30, 95% CI 1.01-5.27; P=0.048), long-term DAPT did not reduce the risk of MACE but instead increased the risk of bleeding among patients with stents, regardless of diabetes status [24].

Besides, a recent meta-analysis involving eight studies, and 12,665 participants observed that the implementation of dual antiplatelet therapy for ≤three months (S-DAPT) in diabetic patients led to a 17% reduction in the risk of net adverse clinical events (NACE) compared to standard-duration DAPT (RR: 0.83, 95% CI; 0.72–0.96). These findings suggest that S-DAPT could represent a safe treatment option for diabetic patients. Interestingly, the analysis indicated that ticagrelor-based S-DAPT was linked with decreased mortality rates [25].

On the contrary, Grodzinsky et al., conducted a study using a real-world PCI registry comprising 2334 patients from 10 hospitals in the USA focusing on bleeding risk in patients with DM, that have been prescribed DAPT following PCI. Their findings indicated that diabetic patients experienced fewer bleeding events during the 1-year follow-up period post- DAPT (RR 0.89, 95% CI 0.83-0.96) compared to non-diabetic patients. Consequently, they concluded that prolonging dual antiplatelet regimen might be advantageous for diabetic patients, considering their heightened ischemic risk [26].

B. DAPT after Complex- PCI

"Complex" PCI, despite the lack of a universal definition, refers to percutaneous coronary intervention procedures that involve challenging anatomical features, such as multiple lesions, heavily calcified lesions, chronic total occlusions, bifurcation lesions, or lesions in small vessels. These complicated interventions encompass more than 30% of the total PCI procedures and often require advanced techniques and specialized equipment to achieve successful outcomes. For this reason, patients undergoing complex PCI face an elevated likelihood of experiencing adverse events, such as mortality, myocardial infarction, and stent thrombosis. Therefore, antithrombotic therapy plays a role in partially mitigating these risks [27-28].

Angelo et al., conducted a meta-analysis involving 31,627 patients across 5 trials, of whom 8,328 (26.3%) underwent complex PCI. Their study aimed to assess the efficacy and safety of short DAPT (one-three months) compared to standard DAPT (≥twelve months) based on PCI complexity. The authors concluded that patients undergoing complex PCI may experience greater benefit and fewer adverse events from P2Y12 inhibitor monotherapy following early aspirin withdrawal compared to standard DAPT. They found that P2Y12 inhibitor monotherapy, in comparison to standard DAPT, was associated with similar outcomes of all-cause death, stent thrombosis, and stroke, with no observed interaction between complex and noncomplex PCI. However, they noted a reduced risk of myocardial infarction in complex PCI (HR 0.77, 95% CI 0.60-0.99, P = 0.042), as well as a significantly decreased incidence of major bleeding events (HR 0.67, 95% CI 0.49-0.91, P = 0.010) with the strategy of short DAPT [29].

Furthermore, Apostolos et al., conducted another recent meta-analysis involving 6275 individuals to investigate the safety and efficacy of a one-month DAPT regimen compared to a longer duration following complex PCI. The study found that shortening DAPT to 30 days after complex PCI did not result in an increased risk of net adverse clinical events (OR: 0.77, 95% CI: 0.52–1.14) or major adverse cardiac events (including mortality, myocardial infarctions, stroke, or stent thrombosis). Additionally, although there was a reduction in pooled incidence of major bleeding, this finding did not reach statistical significance [30].

The established correlation between abbreviated dual antiplatelet therapy and a reduced incidence of major bleeding events, without a corresponding increase in mortality or ischemic events, is reinforced by advancements in biotechnology [13]. These advancements include the development of drug-eluting stents approved for shortening DAPT to 1 month. Additionally, the increasing use and advancements in intravascular imaging techniques such as intravascular ultrasound (IVUS) and optical coherence tomography (OCT) have improved the optimization of percutaneous coronary intervention procedures [31]. Therefore, even in cases of high PCI complexity, abbreviating DAPT may be safe, but optimizing procedures through appropriate imaging and functional tests remains crucial.

C. DAPT following PCI in patients with malignancies

Patients on dual antiplatelet therapy due to CAD who also have comorbid cancer represent a complex subgroup of individuals with an elevated risk of bleeding and ischemia. This subset, estimated to encompass up to 15% of acute coronary syndrome patients, adds further intricacy to the complexity of post PCI pharmacological

management [32].

Research has shown that readmissions due to acute myocardial infarction within 90 days post-PCI are more frequent in patients with comorbid malignancies (12.1% in lung, 10.8% in colon, 7.5% in breast, 7.0% in prostate, and 9.1% overall). Correspondingly, the rate of rehospitalization due to bleeding is also elevated (4.2% in colon, 1.5% in lung, 1.4% in prostate, 0.6% in breast, and 1.6% overall) [33]. The above-mentioned rates may be attributed to the fact that cancer patients present with numerous intricate characteristics. In addition to common cancer-related adverse events such as coagulopathy and anemia, chemotherapy and radiotherapy can induce prothrombotic, vasospastic, and proinflammatory effects in the vasculature. Moreover, the potential for cancer recurrence may necessitate interruptions in DAPT for procedures such as biopsies, surgeries, or resumption of cancer treatment [34-35]. In summary, these factors collectively contribute to the challenges involved in managing patients with both malignancies and coronary artery disease.

According to the 2022 ESC Guidelines on cardio-oncology, the duration of DAPT should be as short as possible, with 1–3 months clopidogrel based DAPT in patients with a platelet count of over 30,000/Ml being proposed as the optimal therapeutic regimen. Besides, the Academic Research Consortium for high bleeding risk recently established a one-month duration of DAPT as the optimal post- PCI duration for patients with active cancer (excluding non-melanoma skin cancer) and a high risk of bleeding. Additionally, a three-month duration of DAPT is recommended for cancer patients deemed non-high risk. In cases of acute coronary syndrome with an elevated risk of ischemia, an extension of DAPT to six months is advised [36].

Finally, the initiation of OCT imaging may help guide discontinuation of DAPT. This is because risk factors for stent thrombosis, such as stent malposition, incomplete strut coverage, and in-stent restenosis, may be visualized. Consequently, low-risk patients who can safely discontinue DAPT and proceed with essential cancer-related surgeries or procedures can be identified [37].

Overall, in this vulnerable patient population, a tailored and multidisciplinary approach is paramount to enhance both life expectancy and quality of life for patients.

Future Perspectives

The optimal duration of dual antiplatelet therapy and the most effective agent for subsequent monotherapy have been subjects of recent scrutiny in clinical studies. Following encouraging outcomes from studies advocating for shortened DAPT durations, with subsequent administration of a P2Y12 inhibitor alone, interest has grown in exploring the complete omission of aspirin immediately after percutaneous coronary intervention. The recently published ASET trial [38], a pilot investigation conducted by Kogame et al., examined the safety and feasibility of prasugrel monotherapy following successful everolimus-eluting stent implantation in a carefully selected cohort of patients with low anatomic complexity and stable coronary artery disease. The study yielded positive results, prompting the initiation of larger RCTs to further evaluate this treatment approach. Furthermore, the long-awaited STOP-DAPT-3 trial [39] was recently published, comparing single low-dose prasugrel to the standard DAPT strategy in patients post-PCI following an acute coronary syndrome or those at high risk of bleeding. However, the trial's no aspirin strategy failed to reduce major bleeding within 1 month and showed an increased risk of coronary events. Consequently, the ongoing NEO-MINDSET study (NCT04360720) holds promise to provide additional insights into this innovative treatment strategy and elucidate its potential for clinical application [40]. Finally, the T-PASS trial, which focused on ACS patients treated with new-generation bioresorbable polymer sirolimus-eluting stents (BP-SES), demonstrated that a short-term DAPT strategy of less than one month followed by ticagrelor monotherapy was both non inferior and superior to the conventional twelve-month ticagrelor-based DAPT regimen. This superiority was evidenced by a significant reduction in bleeding complications in the short-term DAPT group, while the risk of major adverse cardiac and cerebrovascular events remained similar between the two groups [41]

A significant revolution in the field of DAPT may be on the horizon with the introduction of several new antiplatelet agents. Among these agents currently under clinical development for use in patients undergoing PCI are selatogrel, a reversible non-thienopyridine P2Y12 receptor antagonist administered subcutaneously [42]; revacept, an intravenously administered inhibitor of GPVI, the major platelet collagen receptor [43]; and RUC-4, a GPIIb/IIIa inhibitor with a novel mechanism of action designed for intramuscular administration [44]. These innovative antiplatelet agents hold great promise for improving outcomes and reducing complications in patients undergoing PCI.

CONCLUSION

In conclusion, the future of DAPT lies in its continued evolution, driven by advancements in medical science and technology, as well as a deeper understanding of patient-specific factors. By embracing evidence-based approaches and innovation, clinicians can optimize treatment strategies and ultimately improve the prognosis and quality of life for patients undergoing PCI.

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Understanding Human Hydrocephalus: Insights into Genetics and Molecular Mechanisms

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Abstract

Hydrocephalus is a progressive neurological disorder associated with abnormal cerebrospinal fluid (CSF) flow resulting in an active distention of the ventricular system. Three main types of hydrocephalus have been described, including non-communicating or obstructive, communicating with reduced CSF absorption, and communicating hypersecretory. Despite common shunting procedures used for symptomatic treatment of ventricular enlargement, patients still develop symptoms, indicating the complexity of the pathogenesis of hydrocephalus, suggesting that the disease is not a mere disturbance of circulative procedure. This review aims to present the genetic and molecular aspects of human hydrocephalus associated with congenital disorders, such as X-linked hydrocephalus, the most common form of genetic hydrocephalus linked with L1-CAM mutations, and other complex pathologies including common syndromes such as primary ciliary dyskinesia and Dandy-Walker malformation. Reevaluating existing hypotheses in hydrocephalus research, such as the cilia hypothesis and glymphatic flow disruption and comprehending novel data, including downregulation of Aquaporin 1 (AQP1), a water channel involved in CSF production, and the interconnection between neurogenic defects and tissue biomechanics will pave the way for improved diagnostic and therapeutic strategies for human hydrocephalus.

Key words: *Congenital/developmental hydrocephalus; cerebrospinal fluid; brain development; glymphatic flow; AQP1*

INTRODUCTION

CSF production and drainage

Cerebrospinal fluid (CSF) is an ultrafiltrate of plasma accumulated in the ventricular system and the subarachnoid spaces of the cranium and spinal column. Adults acquire roughly 150ml of CSF, with a diffusion of 125ml in subarachnoid spaces and 25ml in the ventricles. Approximately 20% of CSF is primarily produced by modified ependymal cells, the choroid plexus, within the lateral, third, and fourth ventricles by percolation of plasma. These ependymal cells are highly specialized, simple, cuboidal epithelium connected with tight junctions creating the blood-CSF barrier, responsible for filtration of CSF, allowing passage only to ions and small molecules such as vitamins. CSF renewal is a persistent procedure manifesting four to five times repetitions per 24 hours in adults. This process is crucial for the proper functioning of the brain since CSF contributes to nourishment, waste removal, and protection of the brain. An impairment in CSF renewal would promote the aggregation of waste metabolites leading to aging and neurodegenerative diseases [1].

CSF passes from the lateral ventricles via the in-

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terventricular foramen (of Monro) to the third ventricle and subsequently to the fourth ventricle via the cerebral aqueduct or aqueduct of Sylvius. It exits the fourth ventricle, entering the basal cisterns, through the two lateral foramina of Luschka and the foramen of Magendie. Some of it infiltrates the subarachnoid space around the spinal cord. Arachnoid granulations, which are shaped by the arachnoid mater, project into dural venous sinuses, especially the superior sagittal sinus, and are responsible for CSF absorption. Lastly, CSF is assimilated into the venous sinuses ("bulk glow"), reentering into the systemic circulation [1] (Figure 1).

Hydrocephalus Definition and Classification

Hydrocephalus refers to a Central Nervous System (CNS) condition characterized by a surplus of CSF accumulation in the ventricles. In early 1913, Dandy was the first to propose a classification of hydrocephalus as communicating and non-communicating (obstructive). Since then, many more classifications have been demonstrated. Specifically, there are three main types of hydrocephalus: non-communicating/obstructive (↓ CSF absorption), communicating (↓ CSF absorption), and communicating hypersecretory (↑ CSF production). However, some special forms of hydrocephalus have also been described: normal pressure hydrocephalus (NPH), entrapped 4th ventricle, arrested hydrocephalus, and hydrocephalus ex vacuo [2-5].

Specifically, non-communicative or obstructive hydrocephalus is established by a blockage in CSF pathways, from the ventricles to the subarachnoid space. Different types of brain tumors constitute some of the most common obstructions at the foramina of Monro, cerebral aqueduct of Sylvius, fourth ventricle, median foramen of Magendie, etc. Other acquired causes that could lead to obstructive hydrocephalus are space-occurring lesions, such as brain abscesses, and clots due to hemorrhage. Furthermore, some congenital diseases are leading to obstruction in the ventricular system. These include Arnold-Chiari malformation, Dandy-Walker malformation, intrauterine infections, such as congenital toxoplasmosis, colloid cyst obstructing the

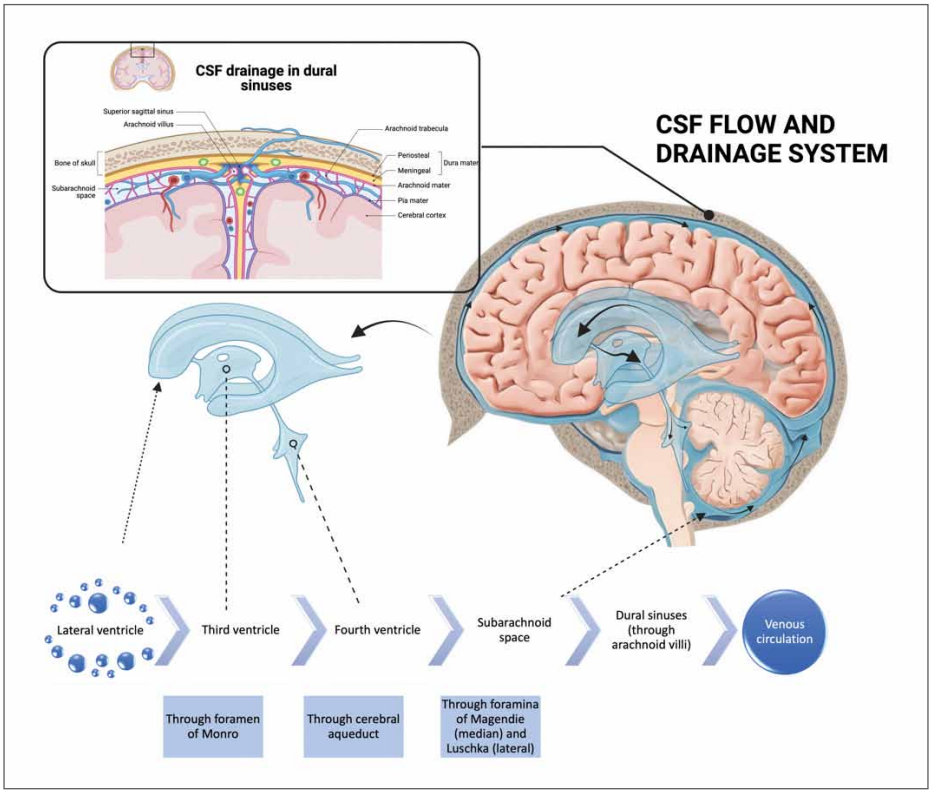

Figure 1. Schematic illustration of CSF flow and drainage. The CSF is produced by the choroid plexus, flows through the ventricles to subarachnoid space through the foramina of Luschka and Magendie, where it is subsequently drained from the dural venous sinuses through arachnoid villi. (Created with BioRender.com)
CSF: Cerebrospinal fluid

interventricular foramen of Monro, and congenital stenosis of the cerebral aqueduct of Sylvius [2-5]. Communicating hydrocephalus occurs when the flow of CSF is obstructed in the arachnoid villi or subarachnoid cisterns after it exits the intraventricular system. This form of hydrocephalus is termed communicating because the transit passages between the ventricles remain open allowing CSF flow within the ventricular system. The most common cause of communicating hydrocephalus is infectious diseases of the central nervous system, such as meningitis or cysticercosis, leading to inflammation of arachnoid villi and eventually their abolishment. Other common causes are post-hemorrhagic, such as subarachnoid and post-intraventricular hemorrhage, and post-Traumatic Brain Injury (TBI) changes. Congenital absence of arachnoid villi could also more rarely lead to communicating hydrocephalus [2-5]. Hypersecretory communicating hydrocephalus is prompted by CSF overproduction. It is presumptively caused by choroid plexus papilloma or more seldom carcinoma. These tumors appear more often in childhood. Moreover, inflammation related to the choroid plexus can lead to hypersecretory communicating hydrocephalus [2-5].

Normal pressure hydrocephalus NPH was first described in 1965 and is a form of chronic communicating hydrocephalus, characterized by normal intracranial pressure (ICP) or slightly elevated. It can be idiopathic (iNPH) appearing most commonly in elderly people or "secondary NPH" due to chronic obstruction of CSF flow. It characteristically occurs with the distinct Hakim triad of reversible symptoms including progressive gait apraxia, urine incontinence, and dementia [4,5]. Entrapped fourth ventricle is a rare neurosurgical condition which occurs when the fourth ventricle communicates neither with the third ventricle nor with the basal cisterns. It most commonly manifests in patients with chronic lateral ventricular shunting and more seldom in Dandy-Walker malformation, intracranial masses, choroid ventriculitis [5]. Arrested or compensated hydrocephalus is a term used by physicians mostly to describe a form of hydrocephalus usually present at birth, in which there is no progression and the treatment with CSF shunt would be essential only when symptoms of intracranial hypertension occur [5]. Finally, hydrocephalus ex vacuo is often classified as a distinct form of hydrocephalus, although it is not a true hydrocephalus. It is characterized by ventricular distention provoked by cerebral atrophy either by normal aging or by the progression of certain diseases such as Alzheimer's disease, Creutzfeldt-Jakob disease, Huntington's disease, and TBI [5].

Epidemiology

The estimated prevalence of hydrocephalus in the general population is 1-1.5%. Communicating hydrocephalus is more common than non-communicating hydrocephalus. The incidence of congenital hydrocephalus is approximately 0.9-1.8/1000 births [5]. It is increasingly apparent that genetic factors play a fundamental role in the pathogenesis of some cases of hydrocephalus. In approximately 40% of incidents with hydrocephalus, the pathogenesis is associated with molecular and genetic variations [6]. In this study, the clinical entity of hydrocephalus will be approached regarding the perspective of the molecular etiology of the subsequent CSF accumulation rather than an anatomical viewpoint of hydrocephalus pathophysiology.

I. Unraveling the genetic and molecular profile of hydrocephalus

According to accumulating evidence, the pathogenesis of hydrocephalus is linked with many molecular changes. This phenomenon pertains to a multitude of clinical entities leading to congenital/developmental hydrocephalus which might manifest in the early years of life or later in adolescence or adult life but is not caused by acquired conditions, such as subarachnoid hemorrhage or meningitis. The molecular landscape underlying the pathogenesis of hydrocephalus unfolds in 7 primary axes. The molecular classification of congenital hydrocephalus is as follows: X-linked hydrocephalus with congenital aqueduct stenosis (AS), neural tube defects (spina bifida), Dandy-Walker syndrome, holoprosencephaly, primary ciliary dyskinesia and other ciliopathies, nonsyndromic autosomal recessive hydrocephalus, miscellaneous (less common syndromes) [7-9]. Table 1 presents a comprehensive summary of the main genes responsible for congenital/developmental hydrocephalus.

X-Linked hydrocephalus

The most common form of genetic Hydrocephalus (1/30,000) is linked with a gene mutation in the L1-CAM neural cell adhesion molecule on chromosome X [10-13]. L1-CAM is a glycoprotein, a member of the immunoglobulin-like CAM family, which is an important contributor in neural adhesion, migration, morphology, and growth, mediating cell-cell adhesion [12]. The following genotypic alterations for L1-CAM protein have been reported: Class I: mutations in the cytoplasmic domain of the protein, Class II: mutations in the extracellular domain, Class III: premature stop

Table 1 *A molecular classification of congenital/developmental hydrocephalus.*

Disease	Broad etiologic category	Specific genetic associations
X-linked hydrocephalus with congenital aqueduct stenosis	X-linked hydrocephalus	L1-CAM
CRASH syndrome		
Fried-type syndrome		AP1S2
Myelomeningocele	Neural tube defects	Genes related to neural tube development, ciliary beating
		Genes related to the folate-homocysteine metabolic pathways
Dandy-Walker malformation	Dandy-Walker malformation	Chromosomal abnormalities in 2q, 5p, 8p, 9p, 13q, 16q, 17q
		Gene mutations: POMT1, POMT2, POMGNT1, FKRP, FKTN, ISPD, LARGE
Holoprosencephaly	Holoprosencephaly	Trisomy 13 (most common) and trisomy 18
		Mutations in 7-dehydrocholesterol reductase as well as SHH, ZIC2, SIX3, TGIF etc.
Primary Ciliary Dyskinesia	Ciliopathies	Genes related to ciliary structure and function, e.g. DNAH11, NEK10 and GAS2L2
Other motile ciliopathies		CCNO and MCIDAS mutations
Nonsyndromic autosomal recessive hydrocephalus	Nonsyndromic autosomal recessive hydrocephalus	Variations of MPDZ and CCDC88C genes
Joubert syndrome and Meckel syndrome	Hydrocephalus plus obstructive arachnoid cyst	CC2D2A gene mutations
Phelan-McDermid syndrome		22q13.3 deletion
Noonan syndrome	RAS-opathies	Mutations in RAS pathway (e.g. NF1, BRAF, KRAS, PTPN11)
Cardio-facio-cutaneous syndrome		
Costello syndrome		
Megalencephaly syndromes	Megalencephaly syndromes	Mutations in genes involved in the PI3K-AKT pathway
Craniosynostosis syndromes	Craniosynostosis syndromes	Mutations in fibroblast growth factor receptor (FGFR) genes
VACTERL-H sequence	VACTERL-H sequence	Mutations in the FANCB gene

codon in the extracellular domain and loss of function, Class IV: mutation in noncoding regions [13]. An association between ventricular dilation and mutation class of L1-CAM has been found. Additionally, L1-CAM mutations are responsible for CRASH syndrome, a very rare inherited disorder characterized by corpus callosum hypoplasia, retardation, adducted thumbs, spastic paraplegia, and hydrocephalus which led to the acronym CRASH syndrome [14]. Another X-linked hydrocephalus disorder is called Fried-type syndrome, caused by AP1S2 encoding gene mutations [15]. This disorder is manifested with hydrocephalus, intellectual disability, and iron deposition in the basal ganglia. Moreover, in some cases, aqueduct stenosis and/or fourth ventricular or retrocerebellar cysts have been reported [10].

Neural Tube Defects

Neural tube defects occur due to incomplete closure

of the neural tube during embryogenesis. Spina bifida aperta (Myelomeningocele), caused by failure of closure of the caudal neuropore, is the most frequently observed defect [13]. Hydrocephalus occurs in 80-90% of these cases and may be caused by genes related to neural tube development or genetic mutations that affect ciliary beating and ependymal cell polarity [13,16]. Nevertheless, multiple gene mutations and environmental factors seem to contribute to its development while sufficient intake of folate during pregnancy is shown to decrease the occurrence of the disease. Indeed, risk factors include genetic variants of single genes that encode the metabolism of folate-homocysteine or gene-related interactions amongst different folate molecular pathways [16-19]. Notably, in utero closure of spina bifida lowers the occurrence of post-natal hydrocephalus and the need for surgical management [20,21] whereas positive impact on Chiari II malformation and neurological deficits has been also observed [20,21].

Dandy-Walker malformation

Dandy-Walker malformation (DWM) is a common congenital malformation (1/25000-35000) which consists of hypoplasia and rotation of the cerebellar vermis, enlargement of the posterior fossa and fourth ventricle and rostrally shifted lateral sinus, tentorium and torcula herophili [13,22]. In addition to these classical findings, DWM is characterized by additional abnormalities and malformations of the CNS, including agenesis of the corpus callosum, heterotopia, occipital meningocele, visual deficits, epilepsy, schizencephaly and glial heterotopia [22,23]. Genetic and environmental factors seem to contribute to its development. At least 18 types of chromosomal abnormalities e.g., abnormalities in 2q, 5p, 8p, 9p, 13q, 16q, 17q but also mutations in several genes [Protein O-mannosyltransferase 1 (POMT1), Protein O-mannosyltransferase 2 (POMT2), protein O-mannose beta-1,2-N acetylglucosaminyltransferase (POMGNT1), fukutin-related protein (FKRP), Fukutin (FKTN), Isoprenoid synthase domain-containing gene (ISPD), LARGE Xylosyl-And Glucuronyltransferase 1 (LARGE)] have been associated with this disorder. Also, fetal viral infections (CMV, Rubella), maternal diabetes, maternal use of warfarin and alcohol use during brain development have been linked to DWM [10,13,23,24]. Hydrocephalus develops in more than 80% of DWM patients [23].

Holoprosencephaly

Holoprosencephaly (HPE) is a brain malformation in which the prosencephalon (embryonic forebrain) fails to separate into two different lobes (3-4 weeks of gestation) caused by neural differentiation abnormalities [25]. It appears in 1/10,000 births and is usually accompanied with hydrocephalus, DWM and craniofacial abnormalities [25]. According to the grade of separation, this condition is categorized as a lobar, semi-lobar and lobar holoprosencephaly [13]. Holoprosencephaly has been shown to be linked with chromosomal abnormalities in 25%-50% of cases including trisomy 13 and 18 with trisomy 13 most common. The other 50% of cases may be associated with maternal diabetes, alcohol use and smoking, anticonvulsant drugs, retinoic acid, CMV infection of the fetus, hypercholesterolemia, and mutation in 7-dehydrocholesterol reductase and a minimum of 16 other HPE-associated genes e.g., Sonic Hedgehog gene (SHH), Zic family member 2 gene (ZIC2), SIX homeobox 3 gene (SIX3), TGFB induced factor homeobox 1 gene (TGIF1) [13,26,27].

Primary ciliary dyskinesia and other ciliopathies

Primary ciliary dyskinesia (PCD) has an incidence of 1/15-30,000 births. Hydrocephalus may be present among other pathologies, like situs inversus congenital heart disease, polysplenia, or asplenia [28-30]. This disorder is caused by cilia dysfunction in which ciliary and flagellar motility or orientation is affected. Motile cilia play a crucial role in fluid flow and are composed of a basal body in order to anchor to the cell membrane and an axoneme made by 9 + 2 microtubules (a ring of nine doublets, and a single central pair). During fetal neurodevelopment, there is another type of motile cilium made of a 9 + 0 axoneme which activates a signaling cascade for the establishment of left-right sidedness and body laterality. Furthermore, dynein motor proteins play a critical role in cilia motility, composing inner and outer dynein arms on the outer microtubule doublets [28,29]. Being genetically heterogeneous, primary ciliary dyskinesia is primarily an autosomal-recessive disease while autosomal-dominant and X-linked type has been found. About 50 genes are associated with this disorder and most of the genes encode proteins that are responsible for axonemal motors, structure and regulation or assembly and preassembly of cilia e.g., dynein axonemal heavy chain 11 gene (DNAH11), NIMA related kinase 10 gene (NEK10) and growth arrest specific 2 like 2 gene (GAS2L2) [29]. However, Duy et al., (2022) have observed that the occurrence

of hydrocephalus in human PCD was found to be low (1.3% in some cases) [31]. It has been reported that hydrocephalus appears primarily as a result of reduced ependyma cilia beating in narrow paths and secondary changes in CSF production via altered ependyma and choroid plexus microenvironment [32]. Other motile ciliopathies are associated with biallelic mutations in Cyclin O (CCNO) and Multiciliate Differentiation and DNA Synthesis Associated Cell Cycle Protein (MCIDAS), important proteins for centriole production. Interestingly, MCIDAS mutations demonstrate higher hydrocephalus incidence. Meanwhile de novo single mutations in Forkhead box J1 (FOXJ1), a transcription factor for cilia gene expression, result in motile cilia number reduction and hydrocephalus, laterality defects and recurrent respiratory infections in fetus [29].

Nonsyndromic autosomal recessive hydrocephalus

The 2%-11% of congenital hydrocephalus cases (2-4% sporadic/11% familial) is nonsyndromic autosomal recessive type of hydrocephalus [33]. Genetic variations of Multiple PDZ Domain Crumbs Cell Polarity Complex Component (MPDZ) and Coiled-Coil Domain Containing 88C (CCDC88C) genes have been identified in severe autosomal recessive types of hydrocephalus. MPDZ encodes Multi-PDZ domain protein 1 (MUPP-1) a tight junction protein and planar cell regulator whereas CCDC88C encodes Dishevelled -associating protein with a high frequency of leucine residues (DAPLE), a dishevelled-associated protein and negative regulator of the Wnt pathway [33,34]. Autosomal recessive hydrocephalus is usually manifested with ventricular enlargement and an interhemispheric cyst, small vermis and dilated posterior fossa [30].

Miscellaneous

Other less common syndromes include: hydrocephalus associated with intracranial arachnoid cysts impairing CSF absorption from meninges, Joubert syndrome and Meckel syndrome: mutations in the Coiled-Coil And C2 Domain Containing 2A (CC2D2A) gene, Phelan-McDermid syndrome: 22q13.3 deletion, RAS-opathies including Noonan syndrome, Cardio-facio-cutaneous syndrome, Costello syndrome: mutations in RAS pathway [e.g. neurofibromin 1 (NF1), B-Raf proto-oncogene (BRAF), KRAS proto-oncogene (KRAS), protein tyrosine phosphatase non-receptor type 11 (PTPN11)], megalencephaly syndromes: most often mutations in genes involving in PI3K-AKT pathway, craniosynostosis syndromes: mutations in fibroblast growth factor receptor (FGFR) genes, VACTERL-H syndrome: primarily mutations in the FA Complementation Group B (FANCB) gene [10,30].

II. New avenues of exploration: modern research perspectives on hydrocephalus

Hydrocephalus presents diverse challenges in modern research. The emergence of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR/Cas9) genome editing has facilitated the creation of transgenic rat models, such as the L1-cam knockout model of X-linked hydrocephalus (XLH), providing insights into the genetic basis of this condition [35]. Additionally, diffusion tensor imaging (DTI) reveals early periventricular white matter tract injury in hydrocephalus, emphasizing the need for advanced imaging techniques. In a study by Emmert et al., (2019) CRISPR/Cas9 efficiently disrupted the L1-cam gene in rats, leading to hydrocephalus and delayed development whereas DTI unveiled significant reductions in fractional anisotropy and axial diffusivity in specific brain regions. The study emphasizes the potential of CRISPR/Cas9 in creating larger animal models, offering avenues for novel surgical and imaging techniques on a larger scale [35]. Further complicating the understanding of hydrocephalus is the intricate interplay between CSF and cerebral blood flow (CBF) dynamics. Mathematical models, such as Marmarou's compartmental model, offer insights into these interactions. Compensatory parameters derived from CSF circulation models aid in diagnosing and managing hydrocephalus [36]. However, further studies, as reviewed by Kazimierska et al., (2012), suggest alterations in CBF dynamics, highlighting the need for comprehensive modeling approaches that consider both CSF and CBF interactions [37].

Interestingly, hydrocephalus is associated not only with genetic mutations but also with a down-regulation of AQP1 expression in choroid plexus epithelium in hydrocephalus models, suggesting a potential role for AQP1 as a regulator of CSF production [38]. Additionally, deficiency of Geminin coiled-coil domain-containing protein 1 (GemC1), a gene which regulates the balance between neural stem cell (NSC) generation and ependymal cell differentiation in the postnatal brain leads to an increased number of NSCs, contributing thus to the pathogenesis of congenital hydrocephalus [39]. Understanding the molecular mechanisms gov-

erning NSC and ependymal cell dynamics is crucial for unravelling the complexities of hydrocephalus. Recent findings by Li et al., (2023) who have investigated tumour-associated hydrocephalus (TAH), a complication of brain metastases, show that mast cells in the choroid plexus disrupt cilia and consequently increase CSF production, contributing thus to TAH [40]. Hence, a novel perspective is introduced regarding the mechanical interactions between mast cells, cilia, and CSF dynamics, shedding light on a previously unrecognized mechanism in hydrocephalus.

Another novel arena of scientific exploration regarding hydrocephalus pertains to alternative routes of CSF clearance and the glymphatic hypothesis, which postulates the existence of a process for the movement of water-soluble substances in and out of the brain, bypassing the blood-brain barrier. It posits that there is a flow of fluid transporting these substances inward through periarterial pathways, passing through the interstitial space, and then exiting through perivenous routes [41]. In that regard, it has been suggested that beyond its neuroprotective role, CSF facilitates glymphatic clearance whereas disturbances in CSF circulation, common in hydrocephalus, might involve glymphatic mechanisms as well, which can be elucidated through state-of-the-art imaging techniques [42]. Current imaging modalities, including PC-MRI and Time-SLIP, provide valuable insights into CSF flow but have limitations. Prospective research areas include comprehensive "rest-of-body" models and imaging modalities focusing on patients to better understand CSF flow disruptions in hydrocephalus [42]. Furthermore, recent findings add another layer to the intricacies of CSF drainage, presenting the nasopharyngeal lymphatic plexus as a major pathway for CSF flow towards deep cervical lymph nodes, indicating that myogenic control of the cervical lymphatics might be involved in CSF outflow regulation [43]. The subsequent interrelation between the intracranial compartment and the extracranial lymphatic system emerges as a novel control mechanism of CSF flow and a possible therapeutic target to tackle the disease.

Tissue mechanics arise as an additional area of interest regarding hydrocephalus formation. Research on the association of CSF dynamics and mechanical properties, in experimental rat models of hydrocephalus, demonstrates that changes in brain tissue mechanical properties are complex and not necessarily associated with increased brain stiffness during ventricular enlargement [44,45]. The mechanical properties of the cerebral cortex vary between different locations (non-homogeneous, anisotropic tissue) and over time, and neural cells sense and respond dynamically to these changes during development [46,47]. In the adult mammalian brain, NSCs are located mainly in the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the lateral ventricle ependymal wall (neurogenic niche). Besides these classical regions, hypothalamic neurogenesis occurring mainly along and beneath the third ventricle wall is well documented [48]. Thus, the shear stress exerted on the cells lining the neurogenic niche (ependymal cells and tanycytes) is maximized in regions residing near ventricles, where CSF is freely flowing, leading to the hypothesis that mechanical cues might regulate stem cell fate and contribute to neural tissue generation. This finding sets the scene for new avenues of scientific exploration for elucidating the interconnection between mechanotransduction and neurogenesis. The link between neurogenesis disruption and hydrocephalus is further illuminated by animal research indicating that the loss of Ccdc85c, a gene implicated in hydrocephalus in humans (nonsyndromic autosomal recessive) as well as mice and rats alike, disrupts ependymal cell development and leads to ectopic expression of immature neuro-glial cells, further consolidating the role of early neurodevelopmental stages in hydrocephalus pathogenesis [49]. Additional research has identified Tripartite motif 71/lineage defective 41 (TRIM71/lin-41) as a key gene in human hydrocephalus, specifically expressed in neuroepithelial cells, and demonstrated its role in compromising cortical neurogenesis and parenchymal-CSF biomechanics, irrespective of primary defects in CSF flow [50]. These data suggests that neurogenic defects and tissue biomechanics contribute significantly to hydrocephalus pathogenesis, indicating a shift in understanding from CSF drainage defects to intrinsic brain anomalies.

Hydrocephalus research thus enables us to explore the prospect of implementing mechanical cues to facilitate in vitro neural differentiation in bioreactors for tissue engineering purposes. It is evident that the multifaceted challenges in hydrocephalus research involve genetic, molecular, mechanical, and physiological aspects as illustrated in Figure 2, necessitating a holistic approach integrating CRISPR/Cas9 technology, advanced imaging techniques, mathematical modeling, and a reevaluation of existing hypotheses,

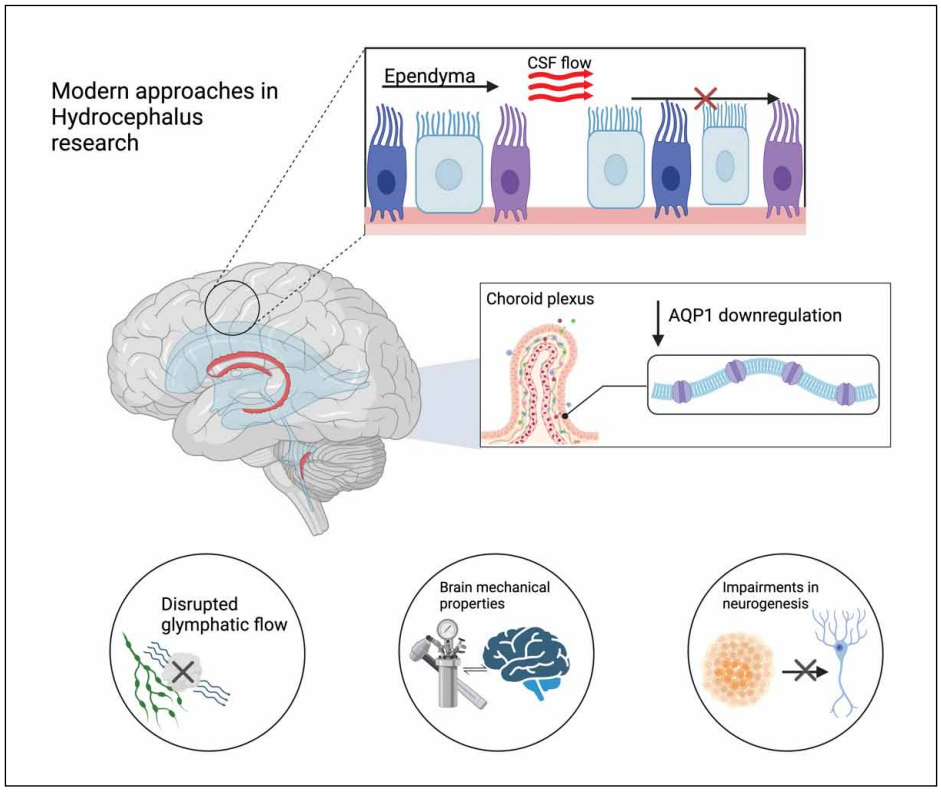

Figure 2. Modern perspectives in hydrocephalus pathogenesis research: Up: Contrasting evidence for the cilia hypothesis, Middle: Alterations in the molecular substrate of ependymal cells, including AQP1 downregulation, Bottom left: The glymphatic flow disruption hypothesis, Bottom middle: Addressing the issue of the mechanical properties of the brain in controlling CSF flow, Bottom right: Impairments in neurodevelopment and stem cell fate determination impacting hydrocephalus pathogenesis. (Created with BioRender.com)
CSF: Cerebrospinal fluid, AQP1: Aquaporin 1

CONCLUSIONS

Hydrocephalus is a clinical entity trademarked by ventricular dilatation caused by a multitude of diseases. Although previously mostly explored under the lens of the anatomical etiology (e.g., obstruction of CSF flow, space-occupying lesions), recent scientific investigation has unveiled an intricate molecular, genetic, and cellular landscape providing a novel perspective towards the pathogenesis of hydrocephalus. Recent findings have shed doubt on established views on hydrocephalus pathogenesis, including the ciliary flow hypothesis while proposing new hypotheses such as AQP downregulation or neural development disruption and capitalizing on the rheological profile of CSF flow as proposed in the glymphatic flow hypothesis. Notably, the mechanical environment in the ventricles seems to be pivotal in regulating the dynamics of CSF-ependyma interrelations, even guiding stem cell fate. In addition, human and animal evidence suggests that motile ciliopathies infrequently cause hydrocephalus in humans, and certain hydrocephalus cases associated with ciliary gene mutations may result from altered neurodevelopment rather than the loss of cilia-generated CSF flow, as ciliary genes are shown to also affect neural stem cell fate. This prompts a reevaluation of the link between motile cilia, CSF physiology, and brain development, crucial for understanding hydrocephalus and related neurodevelopmental disorders. It is thus evident that the etiological substrate underlying hydrocephalus pathogenesis constitutes a kaleidoscope of cellular, molecular, and genetic factors with nontrivial relationships. Further research on the complex interplay of factors leading to hydrocephalus is anticipated to further illuminate the mechanisms of hydrocephalus formation.

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and design; K Birmpas, D Katagi and V Vafeiadis performed the material preparation, data collection, and analysis; V Vafeiadis, K Birmpas and D Katagi prepared the first draft of the manuscript; all authors commented on previous versions of the manuscript; M Assimakopoulou critically edited and reviewed the manuscript; all authors read and approved the final manuscript.

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