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Peer Reviewer of *World Journal of Methodology*, Rodrigo Valenzuela, PhD, Associated Professor, Department of Nutrition, Faculty of Medicine, University of Chile, Independence Av. 1027, Santiago 8380000, Chile. rvalenzuelab@med.uchile.cl

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REVIEW

Challenges to establishing and maintaining kidney transplantation programs in developing countries: What are the coping strategies?

Rabea Ahmed Gadelkareem, Amr Mostafa Abdelgawad, Nasreldin Mohammed, Mohammed Ali Zarzour, Mahmoud Khalil, Ahmed Reda, Hisham Mokhtar Hammouda

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Rabea Ahmed Gadelkareem, Amr Mostafa Abdelgawad, Nasreldin Mohammed, Mohammed Ali Zarzour, Mahmoud Khalil, Ahmed Reda, Hisham Mokhtar Hammouda, Department of Urology, Assiut Urology and Nephrology Hospital, Faculty of Medicine, Assiut University, Assiut 71515, Egypt

Corresponding author: Rabea Ahmed Gadelkareem, MD, Assistant Professor, Department of Urology, Assiut Urology and Nephrology Hospital, Faculty of Medicine, Assiut University, Elgamaa Street, Assiut 71515, Egypt. dr.rabeagad@yahoo.com

Abstract

Kidney transplantation (KT) is the optimal form of renal replacement therapy for patients with end-stage renal diseases. However, this health service is not available to all patients, especially in developing countries. The deceased donor KT programs are mostly absent, and the living donor KT centers are scarce. Single-center studies presenting experiences from developing countries usually report a variety of challenges. This review addresses these challenges and the opposing strategies by reviewing the single-center experiences of developing countries. The financial challenges hamper the infrastructural and material availability, coverage of transplant costs, and qualification of medical personnel. The sociocultural challenges influence organ donation, equity of beneficence, and regular follow-up work. Low interests and motives for transplantation may result from high medicolegal responsibilities in KT practice, intense potential psychosocial burdens, complex qualification protocols, and low productivity or compensation for KT practice. Low medical literacy about KT advantages is prevalent among clinicians, patients, and the public. The inefficient organizational and regulatory oversight is translated into inefficient healthcare systems, absent national KT programs and registries, uncoordinated job descriptions and qualification protocols, uncoordinated on-site investigations with regulatory constraints, and the prevalence of commercial KT practices. These challenges resulted in noticeable differences between KT services in developed and developing countries. The coping strategies can be summarized in two main mechanisms: The first mechanism is maximizing the available resources by increasing the rates of living kidney donation, promoting the expertise of medical personnel, reducing material consumption, and supporting the establishment and maintenance of KT programs. The latter warrants the expansion of the public sector and the elimination of non-ethical KT practices. The second mechanism is

recruiting external resources, including financial, experience, and training agreements.

Key Words: Challenges; Coping strategies; Developing countries; Kidney transplantation; Low resources; Single-center

Core Tip: Kidney transplantation (KT) programs in developing countries are confronted with major financial, sociocultural, regulatory, and political challenges. These challenges result in the delayed establishment or absence of efficient KT programs in most developing countries. They warrant finding different mechanisms to oppose these effects. Maximizing the available resources is mandatory, as represented by investing in living donors and interested medical personnel. In addition, recruiting external resources may be implemented by performing agreements with international academic institutes with expertise in KT and charitable organizations or personnel for practical and financial support.

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INTRODUCTION

The World Health Organization (WHO) classified chronic kidney disease (CKD) as the 10th leading cause of death. CKD is a multi-faceted global health problem. On average, it affects 13.4% of the world's population, and 70% of them are expected to be in developing or low-income countries[1,2].

End-stage renal disease (ESRD) is the terminal stage of CKD. Both the incidence and prevalence of ESRD seem to be increasing worldwide, but the rate of increase is far higher in developing countries than in developed countries. This increase is probably fueled by national underdevelopment, a high incidence of communicable and non-communicable diseases, and poverty[3]. The etiology of CKD has various causes, and it is classically attributed to one of the major contributing pathologies, such as diabetes mellitus, hypertension, glomerulonephritis, interstitial nephritis, and congenital disorders[4]. In developing countries, infections and pollution double the risks of ESRD[2,5]. However, the percentages of ESRD of unknown etiology have been increasingly reported through the last decades, especially in developing countries[6].

Treatment of ESRD constitutes a heavy financial burden, especially in developing countries[3,4,7]. Dialysis and kidney transplantation (KT) are the two modalities of treatment of ESRD. They are inexistent or insufficiently available in developing countries. Even when they are available, the financial affordability of these services remains a major challenge due to the absence of national healthcare insurance coverage in many of these countries. When state support is available, it becomes limited by strict criteria designed for the rationing of dialysis and KT. Hence, in the developing world, renal replacement therapy (RRT) is insufficient, and patients may die prematurely[3,8,9]. Of ESRD patients who need RRT, only 10% receive dialysis or KT[10,11]. In the developed world, almost no one dies because of a lack of RRT, dialysis, and KT units. The majority of cases are in the United States, Brazil, and European countries[12,13].

There has been an upsurge in the number of people accessing RRT globally. However, this is not the case in developing countries like the sub-Saharan countries, where it still accounts for less than 5% of the pool of global RRT[3,14,15]. Moreover, ESRD may lead to a nearly 100% death rate in some countries, such as Guyana, South America, where RRT was not an option until the last two decades[16].

Establishing a KT program in low- and middle-income countries (LMICs) while striving to maintain excellent outcomes and adhere to ethical legal standards is often a formidable and difficult task, requiring expertise and support from developed countries. Maintenance of a KT program is also an extremely complex task in countries with limited resources [5,16-18].

CHALLENGES AGAINST KIDNEY TRANSPLANTATION PROGRAMS

National healthcare systems need to acknowledge and understand the barriers and challenges against RRT to enable KT centers to conduct the necessary interventions towards these disparities and optimize outcomes. There are several different barriers to KT. These barriers can be classified in different ways. Relative to the components of the healthcare process, they may be categorized into patient-related, physician (provider)-related, and system-related. However, individual barriers may lie under more than one category or influence the healthcare system at various levels[19,20]. Hence, they may be better classified based on the nature of the barrier.

Financial and economic challenges

The allocation of small budgets for healthcare systems is a common feature among low-resource countries[4,9]. These financial issues are the main challenge to the availability of proper healthcare facilities. Healthcare systems in developing countries are less funded than in developed countries, where the amounts vary from 0.8% to 4% *vs* 10% to 15% of gross national income, respectively[3,9,21].

Countries in Latin America provide only 3.7% of their gross domestic product (GDP) to healthcare services. They have one of the lowest numbers of health workforce overall. This region has only 16 nephrologists per million population, compared with the minimum target of 20 nephrologists, as recommended by the Latin American Society of Nephrology and Hypertension[2].

Generally, this low expenditure reflects on the availability of RRT modalities. KT seems to be more sensitive to the effects of financial burdens because the latter affects each step of KT. KT costs are pronounced during each step, from preparation to late follow-up[19,22,23]. The financial challenges hamper the establishment and maintenance of KT programs and result in low rates and outcomes of KT due to a lack of the following requirements:

Infrastructure and material requirements for KT units: Infrastructural configurations and logistics are essential requirements for establishing an organ transplantation facility. They are directly based on the economic challenges[17,20,24,25]. In developing countries, it is difficult to construct specific-criteria operative theaters, intensive care units, specific laboratories for immunological workups and pathological studying, services for living and deceased kidney donation, and up-to-the-edge radiological and interventional equipment[20,26-28]. These challenges led to the delayed establishment of KT programs[29-32] or the configuration of inefficient KT programs[17,33-35] in developing countries from different regions[17,34,36] (Table 1).

In South Africa, Davidson *et al*[5] reported that human leucocytic antigen (HLA) typing was initially limited to class 1 (HLA-A, and -B). However, class 2 HLA typing has only been available since 2013 for HLA-DR and HLA-DQ loci. In addition, Single Luminex antigen testing was not available before 2013 and was only introduced to patients awaiting deceased donor KT (DDKT) on the waiting list after 2013. Moreover, B-cell cross-matches were not available at the time of the study[5]. Also, Ahmed *et al*[31] reported on the challenges of KT in Sudan and showed that economic constraints were significant factors that influenced the establishment and maintenance of living donor KT (LDKT) practice. They reported that a few KT centers are available with remarkable insufficiencies in medical supplies and materials that sometimes lead to the discontinuation of LDKT practice. They attracted attention to the lack of medical materials as a common challenge for KT in developing countries. Thus, the financial constraints significantly influenced the supply and price of reagents for immunological tests and other blood tests. Hence, they were obliged to send these samples abroad for analysis as out-of-pocket payments by the patients[31].

Coverage of costs of the KT process: Coverage of all KT costs is available only in 31% of countries worldwide. These costs are needed for preparation, surgery, follow-up, medications (immunosuppressive drugs), and treatment of complications[37].

Lower socioeconomic conditions are a significant factor in the lack of access to KT due to delays in seeking care and the costs of medical evaluations, resulting in late referrals and late waitlisting. Receiving a KT represents a huge financial burden on patients and families[38-40]. In addition, KT recipients have a high potential for out-of-pocket extra expenses for other requirements, such as copayments and travel costs. They may be obliged to resolve their personal or family savings. These factors are more prominent burdens for families with lower socioeconomic conditions. In addition, patients may miss medication visits after KT due to these burdens[19].

In many low-resource countries, health insurance policies do not cover all ESRD patients. Sometimes, this deficiency is compensated by a state-funded policy. However, it is still a form of incomplete financial coverage of the costs. In turn, it fuels concerns about out-of-pocket health expenditure, which is a pressing issue and far exceeds half of total health spending in developing countries[41-43].

In Egypt, the out-of-pocket health expenditure policy represents an economic threat to the viability and sustainability of Egyptian households to the degree of catastrophic health expenditure[44,45]. In a study by Fasseeh *et al*[46], the percent of GDP, which represents the total health expenditure, was almost constant through the last decade, around 5.5%. Out-of-pocket healthcare expenditure contributed to more than 60% of the total healthcare expenditure, followed by government spending through the Ministry of Finance (37%)[46]. This issue has drawn the attention of anthropologists to study it in association with the sociocultural and political views of patients with ESRD[47-51]. Generally, many patients in African countries have to pay for the costs of RRT out-of-pocket, on a continent where widespread poverty is prevalent[3].

In developing countries, many centers may adopt a regimen of no induction immunosuppression, despite the recently introduced induction immunosuppressive agents. This is due to the unavailability and economic issues[36,52,53]. Regarding the maintenance of immunosuppression, patients may not be able to afford the high prices of these drugs. This may result in nonadherence to medication with a high potential for graft loss[54,55].

From the hospital's perspective, an Iranian study of the cost-effectiveness analysis of dialysis and KT showed that the average cost-effectiveness ratio of dialysis was 8.4 times greater than LDKT and 14.07 times higher than DDKT. Hence, the authors recommended increasing kidney donation from both deceased and living resources[56]. In a Brazilian study, savings per patient in DDKT were Brazil Real (BRL) 37000 and BRL 74000 compared to hemodialysis and peritoneal dialysis, respectively. In LDKT, however, savings were even greater: BRL 46000 and BRL 82000 compared to hemodialysis and peritoneal dialysis, respectively. In addition to the advantages in survival and quality-of-life analyses, this benefit characterizes KT as the best clinical and financial alternative in Brazil[57].

However, the costs may be different in other countries, such as Sudan. Elsharif *et al*[58] found that the annual cost of hemodialysis was United States $ 6847. The total costs of the first and second years of KT were United States $ 14825 and

Table 1 Examples of challenges and shortages in the establishment or maintenance of kidney transplantation programs in studies from developing countries

Ref.	Country	Shortage
Budiani-Saberi and Mostafa[33], 2011	Egypt	Commercial living donors care
Haddiya *et al*[24], 2012	Morocco	Lacking human and material resources
Okafor[17], 2016	Nigeria	Absent DDKT
		Lacking human and material resources
Mekdim *et al*[32], 2017	Ethiopia	Delayed program establishment
		In September 2015, the first Ethiopian KT program was launched in Addis Ababa, in collaboration with the University of Michigan
Davidson *et al*[5], 2015	South Africa	Materials for immunological tests: HLA and cross-matches
Ahmed *et al*[31], 2018	Sudan	Materials for immunological tests
		Delayed program establishment: In 2000, it was established with the assistance of visiting teams from England
Naqvi and Rizvi[34], 2018	Pakistan	Organ selling
Guy-Frank *et al*[16], 2019	Guyana	Unavailability of RRT: HD and KT. Only HD before 2007 with limited sessions (high costs)
		Delayed program establishment; started in 2008 with the assistance of volunteer teams from the United States
Bakr *et al*[35], 2020	Egypt	Absent DDKT program
Babloyan *et al*[36], 2021	Armenia	Financial issues for immunosuppressive and antiviral agents
		Irregular deceased KT program; Delayed establishment of LDKT program; launched in 2002, assisted by Belgium, Switzerland, and International Society of Nephrology and Guy's Hospital (London)
Gadelkareem *et al*[30], 2023	Egypt	Delayed program establishment
		Incomplete national KT program

DDKT: Deceased donor kidney transplantation; HD: Hemodialysis; HLA: Human leucocytic antigen; KT: Kidney transplantation; LDKT: Living donor kidney transplantation; RRT: Renal replacement therapy.

United States $ 10651, respectively. However, the total admission days and absences from work were fewer in the KT group, rendering hemodialysis less expensive than KT[58].

Training and promotion of the expertise of medical personnel: Promoting training in KT is a great challenge for the practice of KT. It is usually accomplished through separate training courses, workshops, and specified corners or quotes for the training of healthcare personnel in scientific meetings or congresses. In addition, training grants or stays in centers mastering KT may be offered. However, its financial coverage is creating a problem in developing countries. Despite the complex coordination efforts and recruiting rules, support is mandatory from scientific societies, healthcare administration, and pharmaceutical companies[59].

Specifically, the vascular anastomoses of a KT are time-sensitive, and the inexperience of young surgeons or urologists can negatively impact outcomes. Hence, training in KT surgery and medicine is fundamental. Simulating models are used to teach surgical anatomy and operative skills in KT surgery. Training programs using these models are mostly available from centers with a long history and expertise in KT[60-62]. These centers are mostly from developed countries with high costs. However, novel models have been introduced to reduce the cost issues[59,61]. On the other hand, many experiments in developing countries reported the presence of deficient numbers of nephrologists with expertise in KT. The African countries, especially those in the sub-Saharan region, do not have trained personnel for KT[26].

Sociocultural challenges

Socioeconomic and sociocultural challenges are overlapping problems and influence all aspects of the KT process. This spectrum is a mixture of religious, traditional, and ideological challenges[19,51]. These challenges influence the establishment of a KT program through the following axes:

Effects on organ donation: Kidney donation is a principal item in any KT program. This resource is highly sensitive to religious instructions and traditional beliefs. Hence, the challenges are viewed through:

(1) Religion and science: The justification of organ donation is based on a balanced interpretation of scientific facts and religious instructions. Religious reasons may be the motivating factor for organ donation among the population, but at the same time, they may be the reason for refusing to donate organs among others. However, knowing the agreements between religion and law towards encouraging organ donation, 76% of the participants in the relevant studies would

donate their organs[51,63].

In Egypt, although patients with ESRD have the information that any individual can lead a normal life with one kidney, they may be reluctant to allow a family member to be a kidney donor. They may feel guilty that they will receive an unrepayable favor from a relative. Also, they are worried about the potentially harmful effects of donating an organ to a sibling, spouse, or brother. Generally, those patients express their concerns about the safety of the donor based on religious instructions, such as asserting that the body, as a trust from God, was created in God's perfect wisdom[48,51].

And (2) Traditions: Beliefs such as humiliation of the body or effects on fecundity may discourage organ donation. In a study from Syria, the refusal to disfigure a dead body by removing an organ represented the most common reason to refuse organ donation[63]. Anthropological studies have investigated these traditional beliefs about human body dignity and the political effects on biomedical decisions[48,51].

The delayed establishment of DDKT programs in most developing countries is a clear example of the effect of sociocultural factors on KT[4]. Anthropological analysis has been carried out to investigate the causes of the delayed establishment of the DDKT program in developing countries like Egypt. Many sociocultural factors were discussed, including religion, ethical violations, distrusted healthcare system, and the effect of the media and movies that circulate bad stories about organ transplantation[47,49,50].

Effects on equity of benefits and risks: Gender and age rates show discrepancies in the corresponding rates of donors and recipients. Females are major contributors to kidney donation but are minor beneficiaries as KT recipients[30]. Although these discrepancies are known worldwide, they are rich subjects for the anthropological studies that have tried to analyze their root causes in developing countries. These studies addressed the social roots of the female gender role in the protection of the family. They analyzed the social and physiological concepts that put the mother or the wife in the position of the person who should do everything for the family without waiting for rewards from the other members of the family[47,64].

The balance between the rates of related and unrelated LDKTs in developing countries is another sociocultural challenge. Unrelated LDKT seems to occur more commonly in the private sector than in the governmental sector[35,65]. There is no doubt that social factors are not the only contributors to the increasing unrelated LDKT in the private sector, where many organizational and legislative issues underlie this imbalance[66,67].

Effects on the regular follow-up: Low sociocultural standards are associated with improper post-KT management and outcomes. The latter may be due to noncompliance, unhealthy hygiene, misunderstanding of instructions, *etc.* Noncompliance with medication is common among KT recipients[68,69]. It may result in more than 35% of documented graft losses and failures. Specifically, compliance with immunosuppressive medications and lifestyle modifications is essential for the maintenance of a successful KT[68,70].

Patients with non-adherence to follow-up visits have 1.5 times the risk of acute rejection. In addition, combined medication and appointment non-adherence result in a higher risk of graft loss compared to those who have individual non-adherence[69-71]. The reasons for noncompliance include low financial resources, a lack of perception of the severity of consequences, health and religious beliefs, a lack of social support, and medication complexity or adverse effects[69,72].

Low interests and fading motives for kidney transplantation

Owing to the complex challenges of maintaining an integrated healthcare system in developing countries[5,20], there are common attitudes to prioritize the management of patients with other common comorbidities over the introduction of advanced surgeries such as KT. These attitudes might promote the shift of focus of the healthcare system and doctors away from KT. Hence, there are limited numbers of KT physicians and surgeons in those countries, especially in the public or governmental sector. Significant numbers of physicians and surgeons shift to the private sector, immigrate to high-income countries, or drop the profession, seeking high financial rewards. It has been reported that more than 60% of healthcare services are provided *via* the private healthcare sector[73]. This might be attributed to:

The high medicolegal responsibilities: KT centers, including the personnel, are under regular monitoring and review by different agencies to maintain high performance and provide proper care for patients accessing KT. Then, the complications are highly scrutinized, and strict actions may be taken against transplant centers with below-expected outcomes[5,20,74].

Intense psychosocial burdens under the current healthcare systems: Generally, healthcare systems in developing countries are commonly shared between the governmental and private sectors. This construction represents a license for clinicians to work in both sectors, with competitive attitudes towards the financial rewards that are more prominent in the private sector[73].

Recipients of KT express their perception of KT outcomes in different ways based on their health status. Disproportionally, they may rank graft loss worse than death[75,76]. The perception of the outcomes is markedly related to the emotional status of those patients[77]. Also, they may be exaggerated by financial issues, such as in paid or private sector practice and improper healthcare systems[50,73]. With the initial practice of KT in developing countries, there are usually remarkable psychosocial and emotional challenges. Low sociocultural levels may be associated with high positive expectations that formulate KT as a cure. This narrow-capacity expectation makes the patients and their families find it difficult to accept any major complications that may threaten the graft or the patient's survival. The most particular event is the graft failure and return of the patient to dialysis. This highlights the importance of pre-KT counseling and post-KT comprehensive follow-up. The intensity of these emotional damages usually does not match the realities of KT as a process of therapy with a large scale of complications[18,77].

From personal experiences, lacking standards of the job description, medical literacy among urologists and other physicians about KT outcomes, and selfish individual expectations of KT physicians and surgeons create non-scientific competition among the personnel. All these issues are discouraging factors for the work in peaceful teamwork, which is a mere requirement for any KT program. These challenges influence the outcome of the program and patient safety. Hence, they warrant the presence of a competent program medical director. The latter should have a well-defined role and high skills to manage all the administrative, regulatory, financial, and educational aspects of a KT program[78].

Limited number of centers for KT: Lack of personnel is a major limiting factor in providing RRT in developing countries. In Africa, only Mauritius has 5 or more nephrologists per million population. About 80% of the total number of nephrologists in Africa reside in six countries: Algeria, Egypt, Mauritius, Morocco, Tunisia, and Libya[3,79]. KT activity is still very low in sub-Saharan countries, and graft and patient survival figures are also lower than reported figures from developed economies. Active KT programs are available in only six countries in the sub-Saharan region[5].

Low productivity and compensation: In comparison to general clinicians, transplant clinicians spend significant time on secondary activities such as medical chart reviews, administrative committee meetings, community coordination, and travel to remote outreach clinics. Although these activities are essential to multidisciplinary care and regulatory requirements, financial compensation may not be sufficient, even in developing countries. This pattern leads to low job satisfaction and threatens to sustain a sufficient KT workforce[80].

In developing countries, these issues are more prominent and are a major cause of the emigration of KT clinicians and a significant reduction in their numbers. This is part of a general phenomenon in developing countries[73].

Complex qualifications and work protocols: KT is classified as an advanced surgical procedure, which warrants the availability of high surgical skills and expertise. The time of the vascular anastomoses of KT and the warm ischemia time are significant indicators. They have been shown to have a direct relationship with the development of important negative outcomes such as delayed graft function, graft loss, and mortality. The regular training rounds or rotations of surgery are mostly insufficient to provide the required levels of experience for young transplant surgeons or urologists [61,81,82]. This complexity of KT procedures may indicate further training programs for practicing with self-confidence and expertise sufficient to guarantee surgical safety for the donors and recipients. Highly skilled surgeons are recommended for these surgeries[61,83].

Low medical literacy about the advantages of KT for ESRD patients

General physicians and nephrologists: There are low levels of sound medical information about the advantages of KT over dialysis, early referral to KT centers, and patient counseling. Differences in the attitudes and perceptions of nephrologists influence their decision to refer patients for KT. Hence, a lack of or late referrals by nephrologists to KT centers represents a barrier to KT[19].

ESRD patients: Those patients may be scheduled for regular dialysis without having a chance to know whether KT is possible to be performed in their locality.

A lower education level is a barrier to KT. College graduates experience three times higher rates of being waitlisted and receiving KT when compared with patients without a high school degree. Lower education levels may be associated with a higher rate of allograft loss. However, they might not be modifiable during the assessment of patients for KT. Physicians should recognize this challenge as a potential barrier to KT[19].

Generally, a lack of patients' knowledge regarding KT is a barrier to access to KT, even in countries that have free universal coverage of healthcare[84]. Many studies have reported that patients with ESRD on dialysis have an overall negative attitude towards KT. The reasons for the negative attitude seem to be related to the lack of knowledge and the underlying depression of the disease itself. The absence of trust in the healthcare system has been reported as an important factor promoting unwillingness to have KT[19,85-87].

Public: They represent the pool of potential kidney donors. The majority of the public has no sufficient knowledge about KT. Many studies from different countries and regions showed low knowledge about KT and organ donation among the public[86,88,89]. In a study by Mohamed *et al*[89] about the knowledge and attitudes toward organ donation and transplantation in Minia governorate, Egypt, only 28% of the students had good knowledge, and 32% of them had poor knowledge of all the tested items. Additionally, 34% of the students had a negative attitude towards organ donation and transplantation[89]. Similarly, a study from Oman included 2125 students and showed that only 34.1% had good knowledge about organ donation and more than 70% had a low attitude[88].

In a questionnaire-based study from Egypt by Afifi *et al*[90], 56% of the participants were aware of the presence of organ donation programs in Egypt. This awareness percentage was not paralleled by a significant improvement in the scores of knowledge about organ donation. However, this score was significantly associated with the educational level. Also, attitudes towards organ donation were significantly improved by the education level[90].

In addition, a study from Syria about the knowledge and attitudes towards deceased organ donation showed that more positive attitudes were found in those with better knowledge about brain death. However, these attitudes may not translate into more willingness to donate organs[63].

Organizational and regulatory challenges

Inefficient healthcare systems: The health system includes organizations and people who carry out actions to promote, restore, or maintain the health of populations. A health system includes leadership and governance, health information systems, health financing, health workforce, medicinal products, vaccines, and technologies, and the delivery of health

services[2]. Proper management delivers satisfactory outcomes regarding the healthcare of the target population. However, improper distribution of resources, low rewards, absence of safety, and financial deficiencies discourage proper outcomes. KT is a sensitive topic that warrants a proper and integrated healthcare system. The absence of the latter results in the inability to establish and maintain national KT programs[73].

Lack of national programs and registries: Globally, only 57% of world countries had a KT registry[37]. In the Arab countries, despite the early start of KT practice in some of these countries, there are still many of them struggling with various barriers to establishing national KT programs[91].

(1) Absence of registries for living donors (LDs): They are the main source of graft in developing countries, representing 80% to 100%. However, these rates are far lower than those in developed countries[4,37]. For example, about 600–650 LDKTs are performed in North African countries per year; most are in Egypt[4]. In the Middle East region, LDKT is the main form of KT also[92].

And (2) Absence of DDKT programs: Despite the established legislation for DDKT in many countries, such type of KT is mostly absent on the practical level in African countries[4,93]. Sociocultural and economic challenges back this defect. Egypt is one of the African countries that have tried to establish a DDKT program, but it is still a hard task confronted with sociocultural and economic burdens. These burdens represented a significant challenge for KT in Egypt[4,35]. South Africa remains the only country in Africa that has a DDKT program, representing the major source of grafts. However, this program has many challenges, such as the human immunodeficiency virus and tuberculosis[5,15]. The latter led to a decrease in the number of DDKTs in the last few years[5,93,94].

In India, organ donation centers are at a very primordial stage. They are almost absent in the North-Eastern region. It seems that policymakers and other stakeholders in a big country like India underestimate the magnitude of the benefits of KT programs to ESRD patients. They implement insufficient national policies to emphasize urgent attention to organ transplantation[95].

Lacking management of living kidney donors: Living kidney donation is more prevalent than deceased donation in developing countries. This practice warrants a proper healthcare system with strict legislation to prevent non-ethical threats. This sparks many medical and ethical issues regarding all the steps, such as allocation, registry, and healthcare after donation. If follow-up care for LDs is still improper in countries with advanced KT systems based on altruistic donation, it is largely absent in countries where financial issues control organ donation. Post-donation healthcare is mandatory not only for altruistic LDs but also for victims of organ trafficking. Those donors are often not candidates for a donation and are subject to poor surgical practices. Such follow-up is essential not only as a basic right but also as an important administrative step to regain public trust in transplants against propagating commercialism[33,92].

(1) Absence of allocation systems: Most developing countries have inefficient KT programs. This means the absence of well-integrated patient referral and organ allocation systems. The most important part of the KT program is the presence of DDKT practice because it warrants establishing an allocation system. Hence, the absence of DDKT in most developing countries may be the main factor of lacking allocation policies. One of the clear examples is the KT program of Egypt, which has been dependent on LDKT practice for about 50 years. Despite the presence of a large-volume KT center like Mansoura Urology and Nephrology Center, there is a clear absence of DDKT[35]. In the absence of a national KT program and an inefficient healthcare system, the LDKT practice may be expanded, resulting in uncontrolled KT practice in the private sector. The latter is dependent on individual referrals of the patients.

Other countries tried to find ways to establish a national comprehensive KT program, such as the Iranian Model[96], the private-public partnership model in Pakistan[7], and the KT program of South Africa[5]. However, all these models are not ideal alternatives to a complete program with both DDKT and LDKT divisions. Specifically, the absence of integrated waitlisting and donor allocation policies is a hallmark of lacking systems, even in countries with early beginning KT practice[5,33-35,37].

And (2) Absence of medical registration systems in most RRT centers: In several African countries, nephrologists have attempted to establish registries, but these attempts have not successfully resulted in regular registries. Registries can be traced only in a few African countries, such as South Africa, Egypt, Tunisia, and Ghana, that have regularly published national data on the provision of RRT[4,97,98]. Unfortunately, this shortage led to the absence of or a lack of accurate statistical references for tracing the outcomes and complications[3,4,21]. There is no doubt that these delays in establishing nationwide registries are underlined by the low economic status of most African countries. However, the hope may still be preserved with the novel directions of performing regional or collaborative registries by scientific associations in parallel to the old registries in other regions, such as the European Renal Association-European Dialysis and Transplant Association Registry[3,98].

Uncoordinated job descriptions and qualification protocols for clinicians in different disciplines of the KT team: A multidisciplinary team in KT is a basic requirement for building an efficient program. This warrants an integrated healthcare system and a well-qualified workforce[20]. On configuring a KT team, however, there may be significant difficulties in choosing suitable personnel, such as clinicians, nurses, coordinators, *etc.* This promotes improperly configured teams and the inclusion of inefficient or unqualified personnel. Despite the clear legislative materials, the absence of precise qualification strategies for the inclusion of personnel (surgeons, nephrologists, or others) in KT teams leads to uncoordinated work and distracts the team from the proper work. In turn, unfavorable outcomes and disputes arise among the personnel within the same team. These disputes may be exaggerated by the individual interests of the team members and generate an unsafe environment for all components of the team and even the whole program. Unless there is proper legislation, a coordinated healthcare system, and oversight regulations, as mentioned above, physicians and surgeons may quit KT programs. The reasons may be bad outcomes, unsuitable work standards, and the absence of safety for the donors, patients, and personnel[20,51].

Uncoordinated on-site guidance and regulatory protocols: In developing countries, if we investigate the personnel in institutes seeking the establishment of KT units about whether they want to participate in such a complex task, there will be positive answers based on their initial enthusiasm. However, this enthusiasm is not sufficient to build or establish a good program. The start is always with LDKT programs because they are easier than DDKT programs[20].

In Egypt, well-designed legislative and scoring schedules are mostly available from the Egyptian Supreme Committee of Organ Transplantation[30,83]. In addition, public institutes and university hospitals are now available in each governorate, and basic resources are mostly available to establish LDKT units or centers. This may refer to the availability of physicians and surgeons at these hospitals being higher than at other hospitals in the governmental sector. However, there are still insufficient numbers of KT centers and an absent DDKT program[30,35].

Then, the question is: What is the problem? Away from the economic and sociocultural factors, the coordination between the supervising healthcare system and the institutional workforce may be missing. In other words, the guidance to implement well-designed legislative rules and schedules on-site is absent. Hence, the description, justification, interpretation, and validation of protocols and mechanisms should be implemented on-site for the establishment of a new KT unit. Detailed events from our experience in establishing a LDKT program were the need to facilitate communications between the supervising authorized personnel and the on-practice personnel. In addition, the adaptation of the old infrastructure and administrative procedures of the health facilities to the new standards of KT requirements, which were mostly not suitable, was another detail to be recognized. Of course, the economic and financial issues have prevented the creation of completely novel infrastructure and procedures. Strict protocols and pure electronic systems were mostly different from the old ones, and their changes were clear examples of the demanding efforts that backboned establishing a LDKT program in Upper Egypt, continuing for the eighth year with promising outcomes[29,30].

Prevalent commercial and paid KT practices in developing countries: There is a universal consensus that ethical principles are mandatory to establish and maintain organ transplantation. In addition, the poor resources and social minorities of some populations should not be in favor of others who could pay[20,34].

North Africa hosts six countries: The Western Desert Republic, Morocco, Algeria, Tunisia, Libya, and Egypt, from west to east. Some definitions also include Mauritania and/or Sudan. KT is mostly performed using LDs, and a significant proportion of KT is performed by unrelated LDs, particularly in Egypt, which creates an ethical debate[4]. Hence, Egypt has become one of these countries stigmatized by KT commercial activities[50,51,66]. The official authorities have carried out many interventions to overcome this stigma and overcome these activities[99,100]. The cause may be attributed to low-quality and slow-flowing KT workups in public or governmental centers, low salaries of KT clinicians and other personnel, loss of trust in the public healthcare system, and socioeconomic factors such as poverty and illiteracy[50,73].

In developed countries, cardiovascular disorders represent the major cause of death among KT recipients. Infections represent a significant factor influencing the outcomes of KT. They are a major problem in developing countries, including viral (such as cytomegalovirus, hepatitis C virus, and HIV), bacterial, and fungal infections[5].

All the previous challenges are contributors to the reduced total outcomes of KT. Unfortunately, these challenges have negative feedback on the whole process: The healthcare system, KT teams, and patients[21,37].

COPING STRATEGIES

Maximization of the available local resources

Increasing the rate of living kidney donation: In developing countries, LDs represent the major resource for grafts. Hence, they represent an expandable source to increase the number of LDKT in these countries[13,20]. There are many mechanisms for maximizing the rate of living kidney donation:

(1) Relaxing the age limits of the related kidney donors: The youngest age for living kidney donation is mostly controversial and ranges between 18 and 21 years. Accordingly, the legal age of consent in most countries is 21 years[101,102]. The debate is usually to avoid the longer cumulative lifetime risk of developing conditions that predispose them to CKD, such as diabetes, hypertension, and obesity. A normal initial LD evaluation is not a guarantee against the potential lifetime risk of ESRD[103]. In Egypt, kidney donation in the age range of 18–21 years is often considered a relative contraindication, where it may be allowed only when the donation is to one of the parents. Also, the opposite is correct: Relaxing the upper limit from 50 to 60 years, allowing parents with an age between 50 and 60 years to donate to their sons or daughters. These relaxations of the age limits expand the age range for donation between the donors and recipients within the family from 21–50 years to 18–60 years. This suggestion may need official approval by the Egyptian legislative authorities.

(2) Donor exchange programs: These strategies are very effective and less costly for the resolution of the immunological barriers. Despite the low costs and LDKT as the main policy, their implementation in developing countries is still significantly low. In Egypt, for example, LDKT has been practiced for about 50 years, but the programs of paired kidney donation have not been implemented. However, the KT community has become more vigilant about the need for these strategies. The rate of decline of willing related potential LDs is more than 80%, leaving major proportions of potential recipients on long-term dialysis or for death[30]. Hence, there are strong recommendations for the introduction of paired kidney donation to the Egyptian LDKT program to reduce the decline of potential LDs[30,100,104].

And (3) Healthcare education programs for the public: Improving education about living kidney donation for both potential recipients and LDs is a promising strategy for increasing rates of LDKT. It helps overcome the improper knowledge and socioeconomic barriers to LDKT[105]. There are many interventions to improve the rate of kidney donation, such as sending a donation letter[106]. Education programs should be tailored to the needs and sociocultural

characteristics of the potential donor and recipient populations[107,108].

Investing in medical personnel: Although the number of experienced personnel is small, the potential number of personnel may be high in developing countries. To motivate recruiting personnel from the national healthcare workforce to participate in KT programs, the following strategies may be beneficial:

(1) Increasing the financial compensation of organ transplant teams: The low salaries or rewards should be increased for medical personnel, specifically those involved in organ transplantation. There are considerable numbers of studies that have searched for the effects and mechanisms of overcoming the financial causes of the shortage of practitioners in developing countries[73].

(2) Well-configured plans for regular training, education, and qualification: Many studies have studied the effect of healthcare education on patients' knowledge about KT. They showed that increasing patient knowledge leads to an increased willingness to be evaluated for KT and eventually receive it[109-111]. Patients with sufficient knowledge about the improved quality of life after KT have five times more willingness to receive KT compared with those with insufficient knowledge[112]. Healthcare education is recommended to reduce the high rates of non-compliance with medications and lifestyle modifications[68].

On the other hand, education of medical personnel in primary healthcare facilities is essential to encourage timely referral to nephrology care and KT centers[19]. Generally, the power of youth represents the versatility of developing countries, such as those in the Middle East. This principle can be applied to the field of organ transplantation, including KT. Hence, the perspectives of young transplant professionals are very important for improving the performance of KT programs in developing countries. On the other hand, the challenges of KT would be transferred to young professionals who are pursuing a career in transplantation. These challenges are related to KT education, training, and the healthcare systems. Scientific societies related to KT represent the school for training and the transfer of the wisdom of the leaders to the young professionals. For example, the Middle East Society for Organ Transplantation Region proposed a platform to mitigate these challenges and increase the recruitment and engagement of young physicians and surgeons in the KT practice[113,114]. Webinars represent an electronic education and teaching approach in many KT societies.

Several studies reported the unavailability of nephrologists and surgeons for building a KT program. To establish their KT programs, in-charge personnel in these countries allowed the training of local surgeons in external institutes or hospitals[16,31,32]. For example, Guyanese surgeons completed fellowship training in abdominal organ transplantation outside the country before establishing the KT program in Guyana. Volunteer KT teams helped in the transition from external to local surgeons. After that, several KTs were performed in collaboration[16].

And (3) Reduction of non-medical and regulatory duties for the medical workforce: Reducing the collateral issues that may distract the teams or exhaust their power is another factor that may help preserve the workforce's duties. In developing countries, medical personnel may be hired to manage non-medical activities, such as investigating regulations and providing logistic duties. These activities should be reserved for specified paramedical personnel[5,20,26].

Reduction of the consumption of medical supplies and medications: KT indicates a long list of medications, which are mainly headed by immunological workups, surgical procedures, and immunosuppressive agents. The latter is a main contributor to the high costs of KT. Decreasing the costs of KT warrants decreasing the consumption of medical supplies and medications whenever possible, but without adding risks to the recipients or donors[20].

Reducing the medication amount mostly reduces the total cost of medications. In turn, the reduction in maintenance therapy helps reduce these costs. Reduction of the doses of tacrolimus may be accomplished by using drug enhancers such as calcium channel blockers and ketoconazole[115]. In addition, reducing the amount of medication may improve or prevent non-compliance[116].

Decreasing the consumption of materials in comprehensive national programs was presented in Pakistan. For example, a reusable dialyzer and the use of generic drugs provided an annual saving of United States $ 5.8 million[25].

Promotion of KT programs and system: (1) Completion and maintenance of national KT programs: Integrated kidney care is a recent strategy to optimize the care provided to patients with CKD. It is to develop synergistic links between the different options of RRT, including conservative treatment and preventive care for people with or at risk for milder forms of CKD[28]. This strategy warrants well-configured registries and consistent guidelines[20].

Tonelli *et al*[28] suggested an infographic framework to support decision-makers in establishing and implementing integrated kidney care programs in LMICs. They represented the components of the program proportional to the associated health gains, clarifying the priority for investment. Generally, interventions that control or prevent the progression of early stages of CKD to ESRD should be the highest priority. These interventions aim at treating primary kidney disease. Within the modalities of RRT, KT should be the highest priority but HD should be the lowest priority [28]. Prioritization of KT over dialysis modalities is attributed to the best clinical outcomes at the lowest cost[116,117]. Regarding the countries that choose to offer RRT, a mix of all three modalities of RRT will often be most appropriate[28].

(2) Expansion of KT programs in the public healthcare sector: Related LDKT is more commonly performed in governmental KT centers[35]. However, unrelated LDKT is more commonly performed in the private sector[65]. Hence, control of the private sector will significantly decrease legislative and ethical violations. The private KT sector should be controlled by standardizing the prices of healthcare services, applying legislative standards, and limiting the profit margin from organ transplantation[20].

Major kidney and liver transplantation centers of the public sector in developing countries could be strengthened to become the flagships in promoting the clean image of transplantation. This should be a model that people can see as an example of service being fair, transparent, and equitable for all who need it. This may be achieved by increasing the financial value of the state-funded cases. In turn, it mandates an extra national financial burden[34]. In Pakistan, a model named Free of Cost was implemented to increase access to RRT and KT for all ESRD patients with a life-long follow-up

and medications. This model represents a form of a national donation to fund RRT services by both the public and governmental partners based on the concept of community-government partnership[25].

(3) Ethical control of living kidney donation: Control of the ethical violation issues of KT is a must to improve the performance and outcomes of any KT program, including the prevention of commercial kidney donation[50,20]. In the absence of public or private commitments to the healthcare of commercial LDs, some organizations may supervise and control such practices. For example, the Coalition for Organ-Failure Solutions (COFS) conducts outreach programs to identify victims of organ trafficking, assess their consequences, and arrange support services. In Egypt, COFS had provisions about the follow-up care of commercial LDs, appropriateness as an ingredient for advancing regulation proposals, and movements to end organ trafficking[33].

And (4) Approaches to overcome paid kidney donation: New approaches to mitigate the effects of commercial KT have been proposed, such as the private-public partnership. The latter, underpinned by transparency, public audit, and accountability, is warranted for effective KT services in developing countries[7].

Governmental reimbursement programs for living kidney donations are more prevalent approaches for legitimizing the payment for organ donation. However, the type of reimbursement programs and the ethical dimension of each type currently represent concerns that warrant studying[118,119].

In a unique but debatable model, Iran introduced a government-controlled market for paid kidney donation. This model, known as the Iranian Model, is the only one of its type in the world, which resulted in the elimination of the waiting lists for the LDKT Iranian program. Iranian researchers have continuously advocated the benefits of this model [96]. However, this model is still not spreading outside Iran, with continuous counter-criticism from the other researchers. It has largely been known that Iranian researchers are often prevented from presenting their model at international transplant conferences and publishing it in transplant journals. However, the late publication of data about this program seems to be positively perceived, with some caution as a disclosure of the vagueness and ambiguity of the Iranian Model [120].

Recruiting external resources

Organizational support from developed countries: These intercontinental forms of assistance and supervision by KT professionals usually take different forms from individuals, international societies, or groups such as the Transplant Links Community (TLC). They come with a sense of responsibility towards the patients with ESRD in low-resource countries. Then, they should have the enthusiasm to lobby and act to change the prevailing unfavorable shortage of services to better levels of establishing sustainable programs of RRT[26]. These forms of external resources can be reviewed under the following headings:

(1) International agreements and cooperation protocols with expert centers: In this form of utilization of external resources, agreements can be held between certain centers in developing countries and developed countries. Also, the agreement may be with volunteer persons, such as transplant surgeons. Examples of this form include an agreement between volunteer KT surgeons from the United States and the Guyanese Ministry of Health to establish a national KT program in Guyana. Funding for this project was based on the US-based Guyanese Americans and the Subraj Foundation [16].

Another example is our experience, represented by an agreement between the Assiut KT Unit, representing Assiut University, Egypt, and the Urology and KT Department, Martin Luther University, Germany[30].

And (2) International charitable organizations supporting KT: Charitable donations fund activities represent an ideal form of external resources that are directly participating in establishing and maintaining KT practice in developing countries. The TLC is a clear example of organizational promotion for the national programs of KT. TLC was founded in 2006 and registered as a United Kingdom charity in 2007[26,121]. The aim is to mentor units of KT in developing countries and to drive skill transfer through the performance of LDKT. The ultimate goal is the program's sustainability.

However, to achieve these goals, the mentoring of these units or centers must consider all relevant aspects of development. In addition, it may take a prolonged period to wean these units from direct external assistance. On the other hand, political support and financial underpinnings are fundamental factors in the sustainability of these programs. These intercontinental forms of assistance and supervision by KT professionals from the developed world, as individuals, international societies, or groups such as TLC, come as an indicator of the responsibility of the developed world to help the developing world improve KT services[26].

International individual financial donations: These forms are not common, and personal relations usually play a vital role in recruiting them.

Efficacy of coping strategies

As reviewed above, the challenges to establishing and maintaining efficient KT programs in developing countries are various, multifaceted, and overlapping with each other. However, the financial challenges are the most prevalent ones and underlie most other challenges. Although the challenges of KT programs may differ by the WHO regions, the financial challenges are common characteristics in most developing countries and regions[121-124]. In parallel, recruiting financial resources and reducing consumption are the most adopted coping strategies. These strategies helped resolve significant burdens in countries such as Egypt, where out-of-pocket expenditures of healthcare are the major approach. KT may not be far different from other healthcare services, because many KT centers recruit financial donations[44,46].

Another characteristic is the prevalence of sociocultural challenges in the Eastern Mediterranean and South-East Asian Regions[121,125]. This represented a complex challenge in many countries, due to the strong association between religious instructions and ethical considerations of organ transplantations. An extensive study of sociocultural issues has been conducted, addressing many factors, such as the levels of poverty, religious interpretations, and political strategies

[47-51]. For example, the stakeholders of healthcare policies in Egypt have achieved significant progress towards a national KT program. In the last decades, they established a legislative base, constructed national guidelines, planned the Universal Health Insurance Program, and implemented strict steps against commercial KT activities[33,46,83,100,102]. Despite its slow pattern, we witness the progress of the KT program in Egypt through the increased number of KT centers and serious steps of a national KT program[30,122].

Different regions of the WHO may show some variabilities in the prioritization of the challenges and the corresponding coping strategies[125,126]. In most regions, the financial and sociocultural challenges are the most demanding and persistent ones (Table 2). However, recruiting external assistance and qualification of KT physicians and surgeons by supervising intuitions or volunteer individuals is a common effective coping strategy among the different WHO regions [121-125] (Table 2). In all regions[121-125], many successful examples have been reported[16,18,26,30].

Table 2 Distribution of the challenges to the establishment and maintenance of kidney transplantation programs in different regions of the World Health Organization

Regions	Challenges ordered relative to significance in each region	Proposed coping strategies
Regions	Challenges ordered relative to significance in each region	Proposed coping strategies
AFR	Financial challenges: Lacking human and material resources[5,17,32], delayed program establishment, and absent DDKT[5,17]	To recruit external resources: Training and qualification of KT physicians and surgeons[18,32], out-of-pocket payment[17,31]
	Sociocultural challenges: Religious and traditional beliefs[17,123]	Insignificant workups[17,123]
AMR	Lacking health workforce: Low number of nephrologists[16]	To recruit external resources: Collaboration with expert centers for training[2]
	Financial challenges: Delayed program establishment [16]	To recruit external resources: Financial support by charitable foundations and public-private partnerships[16], the model of the Integrated Healthcare program [20], and reduction of consumption[20,114]
SEAR & WPR	Lacking legislations: Commercial KT and transplant tourism[125]	Activation of local legislation and Istanbul Declaration[125,126]
	Lacking medical personnel[125]	Overseas KT under governmental supervision[125,126]
	Financial challenges[125]	National insurance coverage programs[125]
	Sociocultural challenges: Lacking DDKT[125]	Increasing governmental services and education programs[125,126]
EUR	Financial issues: Delayed establishment of LDKT program[36]	To recruit external resources: Training and qualification of KT physicians and surgeons[36]
	Political policies and consequences[36]	Establishing a national program[36]
EMR	Commercial and organ selling practices [33,34,66,67]	Establishing effective legislation[20,98], governmental reimbursement[117,118], and creation of novel models: Private-public partnership[7] and Iranian Model[119]
	Sociocultural challenges: Religious and traditional beliefs[122]	Anthropologic studies[47-51], education programs
	Organizational and administrative insufficiencies: Delayed or incomplete establishment of the national KT program[30,35]	National and intercontinental registries[96,97]. Establishing effective legislation[20]
	Financial challenges: Lacking human and material resources[24,31]	To recruit external resources: Training and qualification of KT physicians and surgeons[31]. Reduction of consumption[20,14]

AFR: African region; AMR: Region of the Americas; DDKT: Deceased donor kidney transplantation; KT: Kidney transplantation; LDKT: Living donor kidney transplantation; SEAR: South-East Asian Region; EUR: European region; EMR: Eastern Mediterranean Region; WPR: Western pacific region.

CONCLUSION

The major categories of challenges to KT programs in developing countries include financial, sociocultural, regulatory, and organizational challenges. Delayed establishment or absence of efficient KT programs is the main feature in most developing countries, especially in the African Region of the WHO. Their sub-categories are overlapping and multifaceted. As these challenges may differ among the WHO regions, they warrant finding different coping mechanisms. Maximizing the available resources is mandatory, as represented by investing in LDs, qualification of local medical personnel, and activation of the local and international legislative bases. The latter is mandatory to overcome commercialism and tourism in KT practices. In addition, recruiting external resources is another major coping policy. It may be implemented by performing agreements with international academic institutes with expertise in KT and charitable

organizations with funding and training capacities. In addition, individual agreements with volunteer physicians and surgeons from developed countries may be an alternative approach. Implemented coping strategies showed variable success rates. However, increasing living kidney donation is a good local resource, and agreements with centers in developed countries are a common successful strategy for the qualification of the medical personnel for establishing and maintaining successful KT programs. Many efforts are still needed to overcome these persistent challenges. Focused financial and economic corrections and educational programs in developing countries should be attempted. Consequently, most of these challenges can be reduced. Establishing national and regional registries with well-integrated DDKT programs will not be amenable unless all these challenges have been ameliorated or reduced.

FOOTNOTES

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Country/Territory of origin: Egypt

ORCID number: Rabea Ahmed Gadelkareem 0000-0003-4403-2859; Amr Mostafa Abdelgawad 0000-0002-4336-1573; Nasreldin Mohammed 0000-0002-0232-9497; Mohammed Ali Zarzour 0000-0003-1449-6118; Mahmoud Khalil 0000-0002-2564-5476; Ahmed Reda 0000-0003-3699-5735; Hisham Mokhtar Hammouda 0000-0003-2682-3876.

Corresponding Author's Membership in Professional Societies: Egyptian Urological Association.

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REVIEW

Revisiting the standards of cancer detection and therapy alongside their comparison to modern methods

Piotr Gromek, Zuzanna Senkowska, Elżbieta Płuciennik, Zbigniew Pasieka, Lin-Yong Zhao, Adrianna Gielecińska, Mateusz Kciuk, Karol Kłosiński, Żaneta Kałuzińska-Kołat, Damian Kołat

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Piotr Gromek, Zuzanna Senkowska, Elżbieta Płuciennik, Żaneta Kałuzińska-Kołat, Damian Kołat, Department of Functional Genomics, Medical University of Lodz, Lodz 90-752, Lodzkie, Poland

Zbigniew Pasieka, Karol Kłosiński, Żaneta Kałuzińska-Kołat, Damian Kołat, Department of Biomedicine and Experimental Surgery, Medical University of Lodz, Lodz 90-136, Lodzkie, Poland

Lin-Yong Zhao, Department of General Surgery & Laboratory of Gastric Cancer, State Key Laboratory of Biotherapy/Collaborative Innovation Center of Biotherapy and Cancer Center, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Lin-Yong Zhao, Gastric Cancer Center, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Adrianna Gielecińska, Mateusz Kciuk, Department of Molecular Biotechnology and Genetics, University of Lodz, Lodz 90-237, Lodzkie, Poland

Adrianna Gielecińska, Doctoral School of Exact and Natural Sciences, University of Lodz, Lodz 90-237, Lodzkie, Poland

Corresponding author: Damian Kołat, BSc, MSc, Research Assistant, Teaching Assistant, Department of Functional Genomics, Medical University of Lodz, Żeligowskiego 7/9, Lodz 90-752, Lodzkie, Poland. damian.kolat@umed.lodz.pl

Abstract

In accordance with the World Health Organization data, cancer remains at the forefront of fatal diseases. An upward trend in cancer incidence and mortality has been observed globally, emphasizing that efforts in developing detection and treatment methods should continue. The diagnostic path typically begins with learning the medical history of a patient; this is followed by basic blood tests and imaging tests to indicate where cancer may be located to schedule a needle biopsy. Prompt initiation of diagnosis is crucial since delayed cancer detection entails higher costs of treatment and hospitalization. Thus, there is a need for novel cancer detection methods such as liquid biopsy, elastography, synthetic biosensors, fluorescence imaging, and reflectance confocal microscopy. Conventional therapeutic methods, although still common in clinical practice, pose many limitations and are unsatisfactory. Nowadays, there is a dynamic advancement of

clinical research and the development of more precise and effective methods such as oncolytic virotherapy, exosome-based therapy, nanotechnology, dendritic cells, chimeric antigen receptors, immune checkpoint inhibitors, natural product-based therapy, tumor-treating fields, and photodynamic therapy. The present paper compares available data on conventional and modern methods of cancer detection and therapy to facilitate an understanding of this rapidly advancing field and its future directions. As evidenced, modern methods are not without drawbacks; there is still a need to develop new detection strategies and therapeutic approaches to improve sensitivity, specificity, safety, and efficacy. Nevertheless, an appropriate route has been taken, as confirmed by the approval of some modern methods by the Food and Drug Administration.

Key Words: Cancer detection; Liquid biopsy; Synthetic biosensors; Fluorescence imaging; Reflectance confocal microscopy; Elastography; Cancer therapy; Tumor-treating fields; Oncolytic virotherapy; Nanotechnology

Core Tip: Cancer remains at the forefront of fatal diseases, with an upward trend in incidence and mortality. Conventional methods have many limitations, necessitating the development of novel diagnostic and therapeutic approaches. The present paper reviews conventional and modern methods of cancer detection and therapy to facilitate an understanding of the rapidly advancing field and its future directions. Modern methods are not without drawbacks; there is still a need for new strategies to improve sensitivity, specificity, safety, and efficacy. Nevertheless, some novel techniques have been approved for use in clinical settings, certifying that an appropriate route was taken.

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INTRODUCTION

According to the World Health Organization data from 2019, cancer ranks as the first or second leading cause of death in the majority of countries[1]. In populations with higher levels of economic development, mortality associated with cancer is increasing, but those of stroke and coronary heart disease are on the decline[2]. Despite the large volume of research on innovative and more effective therapeutic methods, new cancer cases and related mortality index continue to increase[1,3]. Many causes have been postulated, including the extension of human lifespan and exposure to carcinogens. In addition, while socioeconomic development has improved the quality of life (better living conditions, infrastructure, and medical care), it may also facilitate cancer detection[4,5]. According to worldwide data from the Global Cancer Observatory (GLOBOCAN), there were an estimated 19.3 million cancer cases and nearly 10 million deaths in 2020[6]. Interestingly, males are generally 19% more likely to suffer from cancer. The most common tumors among them include prostate, lung, colorectal, and liver cancer. Females are most commonly diagnosed with breast cancer but also with colorectal, lung, and cervical tumors[1,7,8]. Nevertheless, lung cancer causes the highest mortality among both genders. Comparably important are neoplasms that occur less frequently but have high mortality rates. Examples include brain tumors, with over 300000 new cases and over 250000 related deaths in 2020. The most common and most aggressive primary intracranial tumor is glioblastoma; 95%-97% of patients survive less than three years[9-11]. According to the newest GLOBOCAN estimates, the global cancer burden independent of tumor type is expected to be 35.3 million cases and 18.5 million deaths in 2050.

Detecting tumors in an advanced state makes therapy less effective and more expensive. Despite their widespread use, conventional therapeutic approaches remain unsatisfactory and may cause many adverse effects[12]. There is arguably a need to review existing standards of cancer detection or therapy in light of modern methods due to the rapid advancement in cancer research, the clinical relevance of data generated from novel methods, unmet needs related to treatment resistance and toxicity, as well as an emphasis on tailoring therapy based on individual patient characteristics and tumor profiles. Therefore, the aim of this paper is to provide a clearer understanding of this rapidly advancing field and its future directions; to this end, it reviews available data on conventional and modern methods of cancer detection and therapy.

CURRENT DETECTION APPROACHES AND STANDARD OF CARE

General practitioners play an important role in cancer diagnosis, as they are usually the first to be consulted by patients with worrisome symptoms. Physicians must correctly interpret the information obtained during an interview and

schedule further tests[13]. The current diagnostic path begins with examining a patient and learning about disturbing symptoms or family history of cancer[14]. This is followed by basic blood tests (complete blood count, creatinine level, electrolyte level, liver function test, tumor markers) and imaging tests (X-ray scan, computer tomography, ultrasound, magnetic resonance spectroscopy) to indicate where cancer may be located[15]. Afterwards, the patient is scheduled for a needle biopsy; however, this can be subject to error due to interobserver variability and sample preparation. The results do not take into account heterogeneity because only a fragment of the tumor mass is sampled. Another limitation of this traditional approach is tissue availability[16]. The following step is an investigation of the cancer stage, carried out by an oncologist shortly after receiving all results to provide appropriate treatment on time[13,15,17]. Chemotherapy, radiotherapy, and surgery are standard therapeutic approaches that can be used in combination[18]. They are aimed at neutralizing the cancer but without an entirely individual approach to each patient[19]. These therapeutic approaches are outlined in consecutive paragraphs.

Chemotherapeutic drugs can be divided into four main classes depending on how they affect the cancer cell. These include alkylating agents (form byproducts with the DNA strand), antimetabolites (inactivate components necessary for DNA synthesis), topoisomerase inhibitors (cause DNA strand breaks), and spindle inhibitors (target microtubules to prevent cell division)[19-21]. Chemotherapeutics are harmful to both cancer cells and normal cells, which negatively affects the quality of life due to myelosuppression, inflammation, nausea, vomiting, diarrhea, fatigue, and infertility. For such therapy to be effective, a considerable amount of time is required, which might not be available[22]. Another limitation is the development of drug resistance, during which cancer cells are no longer sensitive to the administered drug(s), and the treatment becomes ineffective. Tumors become resistant due to the reduction in drug availability, alteration of drug targets, cell death inhibition, epithelial-mesenchymal transition, and oncogenic signaling pathways[23,24].

The second pillar of standard cancer treatment is radiotherapy which is administered to approximately 50% of patients [25,26]. It acts by delivering high-energy radiation to cells[25]. Currently, efforts are being made to minimize the exposure of normal tissues to radiation by using, *e.g.*, four-dimensional computed tomography that allows for accurate mapping of the tumor but requires correct positioning and immobilization of the patient during the procedure[27]. Radiotherapy is often used in combination with surgery to reduce the cancerous lesion before surgical intervention and to eliminate any microscopic changes that may have remained after resection[25]. Typically, radiation is systematically administered *via* external beam radiotherapy; however, it can also be provided by brachytherapy – an invasive procedure delivering a source of radiation close to the cancerous lesion[28]. These methods can be used in combination, which usually improves the results of therapy. Despite continuous improvements, radiotherapy is bothersome for the patient. Side effects can develop during radiotherapy (such as acute mucositis, pneumonitis, bronchospasm, proctitis, radiation cystitis) or occur later (*e.g.*, psychological effects after treatment, radiotherapy-induced hypothyroidism, dysphagia, sexual dysfunction, fertility concerns, and toxicity in different body regions)[28-30].

Finally, the tumor can be physically removed with an appropriate margin. Despite the development of surgical techniques, this strategy does not guarantee improvement in the patient's health and carries several risks[31,32]. Surgical interventions can lead to bleeding and damage to surrounding organs and tissues[33,34]. The trauma caused by surgery triggers both local and systemic inflammatory responses, which can potentially cause wound-healing problems and the development of residual and micrometastatic diseases. Another complication is deep vein thrombosis caused by, *e.g.*, prolonged periods of immobilization, blood vessel compression, and hypercoagulability[32,35,36]. Nevertheless, surgical techniques are being improved to minimize side effects. For example, laparoscopy is much less invasive than traditional open surgery. It is characterized by decreased intraoperative blood loss, expedited postoperative recovery, shorter hospitalization, and improved immune response[37]. Another technique is robot-assisted surgery, in which the surgeon manipulates instruments with enhanced precision using robotic arms controlled by a console; however, this method is more expensive and does not achieve better outcomes than laparoscopic techniques[38,39].

MODERN CANCER DETECTION METHODS

Regardless of the method, it is crucial to initiate diagnosis promptly. Patients diagnosed at an early stage of the disease have a much better prognosis and lower risk of death[17]. Survival rate data from 2012-2018 prepared by the SEER program (Surveillance, Epidemiology, and End Results) show that patients diagnosed with stage I cancer have the highest chance of survival (71%), whereas those diagnosed with stage IV have the lowest (14%). Early cancer detection is hindered by the poor sensitivity of screening tests: Only a small number of tumors are detected in non-invasive and rapid procedures[40,41]. Furthermore, the coronavirus disease 2019 outbreak had a considerable effect on early diagnosis as it limited access to health care and dissuaded potential patients from visiting a doctor[42]. Delayed cancer detection entails higher costs of treatment and hospitalization[43,44]. Therefore, novel and more accurate methods of detecting cancer are needed. Consecutive subsections are dedicated to various approaches for diagnosing and monitoring patients. In addition to relatively new methods such as elastography or synthetic biosensors, we will describe tumor-related molecules that can be evaluated using liquid biopsy, a popular solution to some problems of traditional biopsy. Modern and conventional methods of cancer detection are depicted in Figure 1.

Liquid biopsy

Liquid biopsy holds promise in early cancer detection, prognosis, verifying therapeutic response, and monitoring relapses[45,46]. The method involves collecting body fluids such as blood, urine, saliva, cerebrospinal fluid, and bone marrow for analysis[47]. Liquid biopsy is superior to the traditional approach in various ways[48,49]. The procedure is

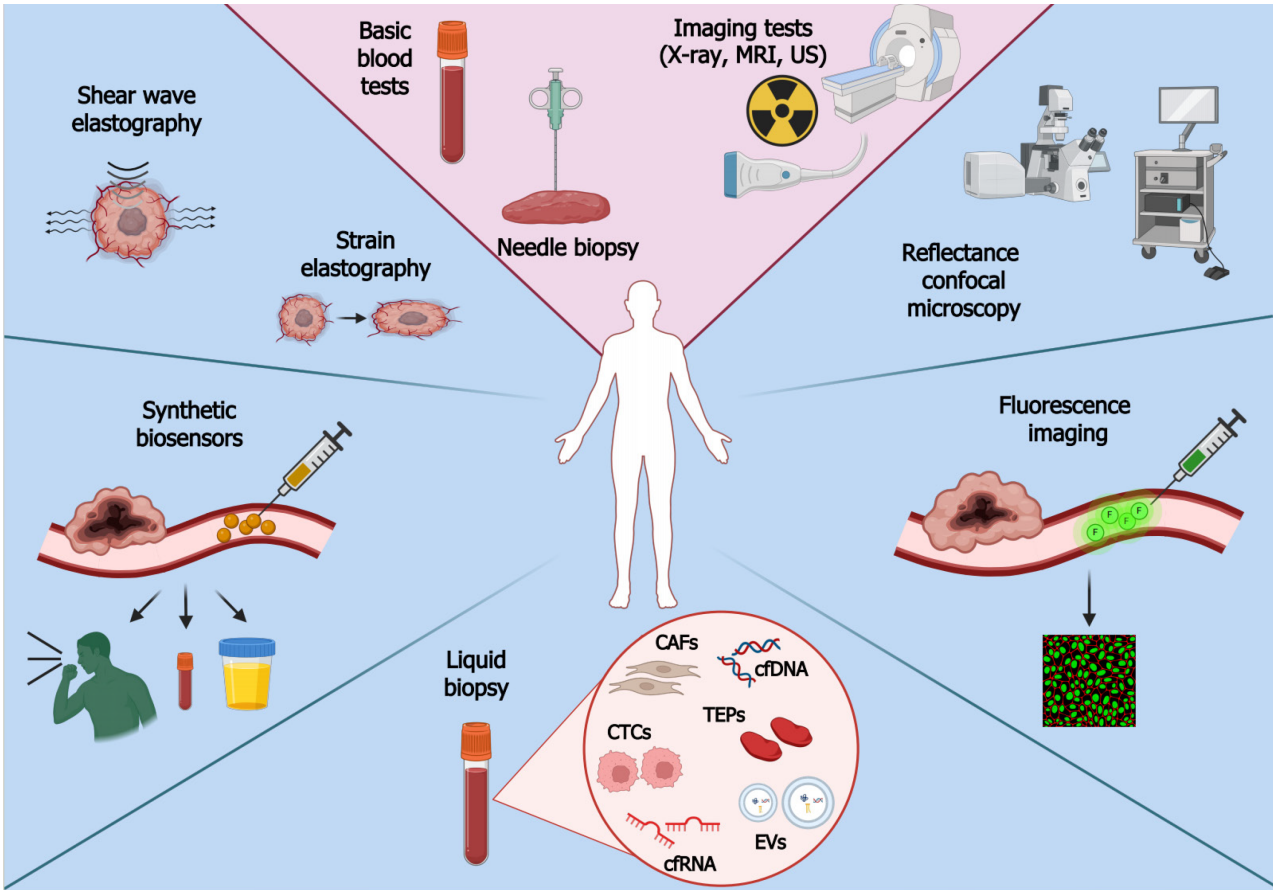

Figure 1 Conventional and modern methods of cancer detection. The diagnostic path begins with learning the medical history of a patient, followed by performing basic blood tests (*e.g.*, complete blood count, creatinine level, liver function test) and imaging tests (*e.g.*, X-ray scan, ultrasound, magnetic resonance spectroscopy) to indicate where cancer may be located. Afterwards, the patient is scheduled for a needle biopsy. Prompt initiation of diagnosis is crucial since delayed cancer detection entails higher costs of treatment and hospitalization. Novel cancer detection methods include liquid biopsy, elastography, synthetic biosensors, fluorescence imaging, and reflectance confocal microscopy. Standards of cancer detection are located in a light pink area, whereas novel methods are placed on a light blue background. Figure created with BioRender. CTCs: Circulating tumor cells; CAFs: Cancer-related fibroblasts; TEPs: Tumor-educated platelets; cfDNA: Cell-free DNA; cfRNA: Cell-free RNA; EVs: Extracellular vesicles; US: Ultrasound (sonogram); MRI: Magnetic resonance imaging.

non-invasive or minimally invasive, which reduces the risks and costs. It offers systemic and homogenous profiles of tumor lesions throughout the body, overcoming challenges related to intra- or inter-tumoral heterogeneity and thus treatment failure. It is also possible to monitor changes in cancer development in real-time. However, the technique also has limitations. One of them is an inability to provide histological evaluation, a crucial aspect for understanding the tissue architecture and cellular characteristics[50]. Secondly, it does not provide comprehensive information about the proteome of a tumor[48]. There is also a need for improvement in the precision and accuracy of detecting biomarkers in bodily fluids, which may be present at very low concentrations. Moreover, the technique is not entirely validated in the clinical spectrum, and reproducibility issues are present due to the use of multiple assays[51,52]. Two main groups of components can be tested. The first includes nucleic acids (DNA and RNA), lipids, proteins, carbohydrates, and various other metabolites or elements. The second one comprises cellular or vesicular components that are released from tumor masses, *e.g.*, circulating tumor cells (CTCs), circulating cancer-related fibroblasts (CAFs), tumor-educated platelets (TEPs), and extracellular vesicles (EVs)[47]. Selected components are described in consecutive subsections.

CTCs: CTCs are components that have detached from the primary neoplasm and entered the bloodstream, which allows them to circulate throughout the body[53]. These cells can be analyzed to determine the ability of the tumor to metastasize[54] or to understand its heterogeneity[55] and allow for identifying prognostic markers[56] or evaluating treatment response[57]. A major limitation of CTC isolation is their limited number in the blood, necessitating the use of sensitive detection methods[47,58]. One such technique is immunomagnetic separation, which uses antibody-labeled magnetic beads to pull down CTCs with appropriate antigens. A widely used antigen is EpCAM which characterizes epithelial tumors, but others are also used, such as ERBB2 and ALK[47,59]. Another approach is to use microfluidic devices with microchannels that allow the size-based separation of CTCs from other blood components. Some of these approaches use antibody-coated surfaces to capture CTCs based on specific markers[54]. The isolation can also be performed using the Ficoll reagent, which separates blood components based on density gradient centrifugation[60]. Since biological cells have diverse electric properties, the components can be separated by dielectrophoresis; however, this is relatively slow and has low throughput[61]. Furthermore, whole genome sequencing can be used to detect mutational variants of CTCs, thus indicating therapeutic outcomes and patient survival. Sensitivity up to one CTC per

milliliter of blood is possible through the use of droplet digital PCR combined with size-based enrichment[62,63]. As technology advances, the capabilities of liquid biopsy for cancer research and clinical applications extend further through the use of new and improved methods for CTC isolation.

CAFs: CAFs influence the tumor and its environment. They regulate proliferation and inflammation *via* the secretion of various growth factors, cytokines, and chemokines[64,65]. The release of proteases such as matrix metalloproteinase by CAFs entails remodeling of the extracellular matrix, leading to invasion and metastasis[66]. Several biomarkers are highly expressed in CAFs and not in other cells; for instance, FAP, FSP, alfa-SMA, CCL11, PDLIM3, AMIGO2, CD70, LOXL2, and UCH-L1[64,65]. Furthermore, cytokeratin and CD31 are considered negative markers since CAFs do not possess endothelial or epithelial characteristics[67]. CAFs may derive from myeloid precursor cells, normal fibroblasts that have acquired genetic mutations, epithelial cells that have undergone epithelial-to-mesenchymal transition, mesenchymal stem cells, or less commonly pericytes and smooth muscle cells[68]. The lack of a specific CAFs-related marker[69] and the diverse origin of these cells can be considered as limitations. In addition, no sufficient exclusion criteria exist among various mesenchymal lineages (such as pericytes or adipocytes), the correlation with specific cancer types/locations is unclear, and few experimental studies have been performed. Consequently, forthcoming studies will concentrate on new biomarkers and methods for selecting CAFs[68]. Nevertheless, the usage of CAFs may be advantageous due to their abundance in the microenvironment, ability to release an ample secretome, and the possibility for modulation instead of depletion, especially since they possess both oncogenic and tumor-restraining functions[70,71].

TEPs: Other components present in the bloodstream of cancer patients are TEPs, in which mRNA expression is influenced by interactions with cancer cells. Such interaction is classified as direct (face-to-face) or indirect (virtual) education. The former is based on a contact between platelets and cancer cells *via* surface adhesion molecules such as mucins, selectins, and integrins, whereas the latter is more distant and occurs *via* extracellular metabolites and enzymes. These interactions alter the transcriptome of platelets, which contribute to cancer progression and metastasis[72]. TEPs regulate various steps of tumor invasion, for example by secreting various growth factors and proteases, which promote the adhesion of the tumor cell to the endothelium and allow disruption of the extracellular matrix. They also help CTCs travel in the blood by protecting them from shear stress and allowing metastatic cells to exit from the blood vessel and form a micrometastatic niche[73]. TEPs can be isolated by gradient centrifugation, flow cytometry, gel filtration, and magnetic bead separation. The predominant approach is gradient centrifugation, enabling quick sample processing and achieving relatively high platelet recovery. Nevertheless, this method introduces notable leukocyte contamination, which can be diminished through gel filtration and magnetic bead separation. However, these methods entail extended duration, increased cost, and reduced yield[74,75]. In cancer research, the use of TEPs offers enhanced sensitivity in detecting cancer cells at an early stage of the disease. Moreover, there is no genomic DNA interference since platelets lack nuclei. Platelets are also continuously exposed to the tumor and its surroundings, which facilitates the exchange of biomolecules with cancer cells[76]. On the other hand, the tumor-derived mutant RNAs in TEPs are only present at relatively low levels, which are often below the detection limits of conventional PCR and RNA sequencing methods, but they can be improved by droplet digital PCR[77].

Cell-free DNA: Cell-free DNAs (cfDNA) are deoxyribonucleic acid fragments with a length of 160-180 bp that circulate freely in the bloodstream[78,79]. They are released into the blood by normal and abnormal cells mainly through apoptosis, necrosis, and active secretion[80]. Within the pool of cfDNA, a subset known as circulating tumor DNA (ctDNA) originates explicitly from tumor cells. Detecting cancer-associated genetic alterations using cfDNA enables early detection of multiple cancers and offers an insight into disease stage and prognosis[81]. ctDNA harbors tissue-specific methylation patterns, facilitating diagnosis and establishment of tumor localization[82]. This method provides a more comprehensive representation of the tumor's genetic landscape than needle biopsy[83]. Furthermore, the ability to serially sample cfDNA allows for longitudinal assessment of dynamic changes in the concentration of cfDNA or tracking clonal evolution, which enables the monitoring of therapy response, acquired resistance, minimal residual disease and recurrence[84]. Technical improvements in detection techniques are needed, particularly given the scant presence of cfDNA and the prevalence of background signals. Moreover, ctDNA assays may exhibit reduced sensitivity in detecting fusion events and copy number changes[85]. It has been proposed that the effectiveness of ctDNA relies on its capacity to reflect the genetic changes identified in the tumor[86]. Because the concentration of ctDNA is the highest in blood[80], it is usually collected in tubes containing the K3 potassium salt of ethylenediaminetetraacetic acid to prevent blood clotting. Special kits have been developed for cfDNA isolation to minimize material loss[87]. Alterations in ctDNA such as translocations, inversions, copy number variations, deletions, and insertions are detected by methods such as digital PCR, cancer personalized profiling by deep sequencing (CAPP-Seq), or tagged amplicon deep sequencing (TAm-Seq)[47,88,89]. CAPP-Seq is ultrasensitive and adopts a hybrid-capture methodology that examines selected genomic regions; it has been applied for diagnosis and molecular profiling, therapeutic response, and detection of postsurgical residual disease[90]. TAm-Seq is an amplicon-based method that utilizes a target enrichment array with barcoded primers to prepare the amplicon library. The two-step amplification design enables the identification of mutations with a sensitivity of over 97% [89]. The enhanced TAm-Seq, denoted as eTAm-Seq, can detect the mutant allele fraction as low as 0.25% with a sensitivity of 94%[91].

Cell-free RNA: Another nucleic acid that can be analyzed by liquid biopsy is cell-free RNA (cfRNA). Among the wide variety of cfRNA, microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are of particular interest in cancer detection and research[92]. Similar to cfDNA, cfRNA originates from cell death, but it can provide more detailed information about the expression signature of cancer cells and their intercellular communication in the tumor microenvir-

onment[93]. Despite this, detection and analysis are complicated by the low abundance and susceptibility of cfRNA to degradation. There is also a lack of standardization of procedures in cfRNA analysis, resulting in inconsistent results across different studies and laboratories[94,95]. cfRNA can be isolated from various body fluids such as serum, plasma, tears, or urine[96]. One method is based on the traditional phenol-chloroform extraction method followed by precipitation. However, this technique is rather prone to contamination, which can be avoided using column-based RNA adsorption[97]. Regarding cfRNA subtypes, the miRNA ranges in length between 20-25 nucleotides and regulates gene expression post-transcriptionally, thus affecting various cellular events. In cancer, they are responsible for sustaining angiogenesis, providing limitless replication potential, and evading apoptosis, but they also inhibit tumor growth or prevent invasion and metastasis[98]. In general, changes in miRNA levels are prevalent among tumors, and their expression is specific to the tissue[43,99]. Some cancer-specific miRNAs have shown promising results in disease detection, such as miR-185-5p and miR-362-5p in breast cancer[100] or miR-451a in pancreatic cancer[101]. Another type of cfRNA is lncRNA, a heterogeneous group of transcripts exceeding 200 nucleotides in length. They are involved in gene expression and various physiological processes but are also known to influence cancer initiation, progression, and metastasis[102]. Accumulating evidence underscores the pivotal roles of lncRNAs in mediating the reprogramming of energy metabolism in cancer[103]. Circulating lncRNAs proved to be useful in detecting tumors of lung (MALAT1, SPRY4-IT1, ANRIL, NEAT1), breast (RP11-445H22.4, H19), gastric (HULC, CUDR, LSINCT-5, PTENP1), liver (AF085935), and prostate (PCA3, LincRNA-p21)[104].

EVs: EVs are lipid bilayer-enclosed particles discharged from a wide range of cell types and present in biological fluids such as blood, urine, saliva, cerebrospinal fluid, breast milk, and seminal fluid. They contain a wide range of heterogeneous molecules that can be used to determine a patient's health status[105]. A few general groups of EVs have been distinguished based on their size and origin, namely microvesicles (100-1000 nm), apoptotic bodies (50-4000 nm), and exosomes (50-200 nm)[106]. The latter have garnered significant interest due to their role in intercellular communication and their potential applications in cancer detection. They carry various biomolecules, including proteins, lipids, and nucleic acids such as miRNAs and mRNAs, reflecting the molecular content of their parent cells. Those deriving from cancer cells often contain a unique cargo of molecules that can serve as potential biomarkers for cancer detection[107]. Exosomes have some advantages over CTCs and cell-free nucleic acids; they are actively released by living cells, and their abundance in biofluids facilitates collection. Their stability, attributed to their lipid bilayers, enables them to circulate even within the challenging tumor microenvironment. This high biological stability allows for the long-term storage of specimens for further isolation and detection[108]. Furthermore, they can be used to identify the present disease by transporting specific molecules derived from parent cells[106]. Unfortunately, isolating these molecules is complicated due to their heterogeneity and small size[108]. Diagnostic accuracy can also be influenced by the physical characteristics of the studied body fluid, such as density and viscosity[105]. Isolation can depend on physical or biological properties since there are centrifugation and size-based techniques[109], or capture-based, polymer-based, and microfluidics-based methods[110].

Synthetic biosensors

The use of endogenous biomarkers detecting cancer at an early stage, *e.g.*, cell-free nucleic acids, proteins, or lipids, is limited due to their relatively quick degradation and low concentrations. However, this can be improved by the use of synthetic biosensors, *i.e.*, exogenous molecules introduced into the body to detect phenotypic changes and enhance cancer-related signals to a conveniently measurable level[111,112]. These synthetics are divided into two main classes, namely activity-based and genetically encoded biosensors.

The first group encompasses protease-activated and small-molecule biosensors. In the protease-activated approach, a biocompatible carrier (*e.g.*, iron oxide nanoparticle or polyethylene glycol) is linked to peptide substrate and cleavable reporter (*e.g.*, mass-barcoded and fluorescent peptide); the latter is detached and detected in body fluid by mass spectrometry or enzyme-linked immunosorbent assay. In contrast, small-molecule probes consist of an enzyme recognition site linked to the substrate molecule and a cleavable reporter such as volatile organic compounds (VOCs) or deuterated metabolites[111,113]. VOC analysis is particularly useful in the early detection of lung cancer, but fewer than 15 compounds (*e.g.*, ethanol, acetone, isoprene) have been found useful. Based on the D5-ethyl-β-d-glucuronide example, it is enzymatically converted into D5-ethanol by β-glucuronidase, an extracellular enzyme secreted by solid tumors. The product is detected from the breath by gas chromatography coupled with high-resolution mass spectrometry[111,112]. Both types of activity-based biosensors described above are administered intravenously.

Genetically encoded biosensors use reporters detectable in biofluids, whose release is influenced by the tumor microenvironment[114]. These synthetic biomarkers are subdivided into vector-based, mammalian cell-based, and bacterial cell-based systems. Each category varies depending on a specific tumor-related characteristic, the "input signal" that is measured, and the method by which this signal is amplified for detection. As the synthetic biomarker is produced in cells of a specific phenotype, this approach reduces the number of false positives caused by background production in healthy tissues. The vector-based system exploits tumor-specific promoters and secretory reporters, which leads to the specific gene expression pattern associated with a tumor. The mammalian cell-based system takes advantage of the metabolic changes in tumor-infiltrating immune cells [typically macrophages but also T-cells, B-cells, and natural killers (NKs)] to induce the generation of a secreted biomarker[115-118]. Bacteria can colonize tumors due to the diminished immunosurveillance and elevated nutrient availability in the necrotic tumor core; the bacterial cell-based system makes it possible to discharge reporters at the tumor site[119]. Vector-based strategies raise concerns about immunogenicity and insertion mutagenesis, which is particularly challenging for early detection applications requiring longitudinal assessment and repeated administrations. Mammalian-based sensors might fail to detect necrotic tumors in high tumor burden scenarios,

likely due to poor infiltration. Additionally, the high cost and complex pipeline make this approach unsuitable for routine screening. Using bacteria for early cancer detection poses safety concerns about the inherent toxicity of bacterial components and the potential for reversion to virulence. It is also unclear if systemically delivered bacteria can colonize all tumor types and nascent lesions lacking a necrotic core[111]. In conclusion, synthetic biosensors are sophisticated and promising but require thorough investigation and standardization.

Fluorescence imaging

Cancer detection through fluorescence imaging (FI) employs diverse optical imaging technologies to enhance the identification of early neoplasia by focusing on unique molecular signatures. This technique is characterized by low invasiveness and considerable specificity and sensitivity. Detecting lesions at an earlier stage leads to enhanced treatment outcomes and lowers treatment expenses by averting the necessity for multimodal care at an advanced disease stage [120]. FI is usually used for the identification of easily accessible tumors, such as those of the oral cavity[121]. Three main types of tumor-targeted fluorescent dyes (TTFDs) are distinguished based on their activation mechanism[122-125]. The first comprises passively-targeted TTFDs that accumulate in some tumors due to their enhanced permeability and retention properties. The second group interacts with an overexpressed tumor-specific ligand, which leads to the endocytosis of the complex and allows for the detection of cancer cells. The last group are activated in the presence of molecules such as enzymes that are predominant in the tumor and its surroundings; the specificity of this TTFD class varies depending on the target, the magnitude of enzyme upregulation, tumor-background ratios and quencher release rates. Limitations of TTFDs include the inability to detect malignant lesions > 2 cm beneath the tissue surface and the tendency to accumulate in some noncancerous tissues[126].

Reflectance confocal microscopy

Another method used to detect cancer is reflectance confocal microscopy (RCM). It facilitates high-resolution imaging of tissues at considerable depths, enabling optical sectioning for the three-dimensional reconstruction of the samples of interest[127]. Produced images are presented in grayscale, with bright (white) structures indicating a higher refractive index compared to their surroundings. Examples of such structures include melanin, keratin, and collagen[128]. RCM is employed to detect skin lesions, which makes it a valuable tool for detecting skin cancers[129]. However, it is not limited to these tumors since it can also determine precancerous and cancerous conditions in the oral cavity or cervix[130,131]. In addition to non-invasiveness, this method has the ability to monitor dynamic processes like blood flow[132]. On the other hand, RCM can be outperformed by biopsy when diagnosing and subtyping primary basal cell carcinoma[133]. Other disadvantages are decreasing image resolution below a depth of 100-150 μm and less robustness in evaluating nuclear details relative to hematoxylin and eosin histopathology[132].

Elastography

Elastography has emerged as a promising and innovative technique for cancer detection, providing valuable insights into tissue biomechanics to overcome the limitations of traditional imaging methods. This approach focuses on assessing tissue stiffness, a characteristic that often varies between normal and pathological tissues[134]. Two main types of this method are strain elastography and shear wave elastography. The first one determines the tissue stiffness by exerting external pressure, which alters tissue dimensions and results in deformation known as strain. Lesions with higher stiffness exhibit less deformation, which manifests in lower strain values and a higher Young's modulus, the latter being a property of materials that measures the tensile or compressive stiffness when the force is applied[135]. Inducing stress depends on the tissue; it can be elicited, *e.g.*, through arterial and respiratory motion in the liver or *via* manual compression in the thyroid[136]. The second type of elastography uses a pushing beam of acoustic radiation force to displace tissue and generate shear waves, which are monitored at multiple off-axis locations. Shear wave speed provides a quantitative estimate of tissue elasticity, with faster propagation observed through stiffer and more contracted tissue [137-139]. Providing real-time visualizations of tissue deformation or measuring the shear wave speed enhances the accuracy of cancer detection. Moreover, elastography can differentiate between benign and malignant lesions, as investigated in breast cancer[140]. Shear waves have been extensively employed in liver imaging, where they assess liver fibrosis and cirrhosis, which are symptoms of liver cancer[136]. Like other techniques, elastography has some limitations. In particularly obese patients with thick subcutaneous fat and ascites, it might be impossible to measure tissue stiffness [141]. The visualization may also return artifacts instead of actual lesions. Nevertheless, integrating elastography into routine clinical practice holds promise for enhancing the precision and efficiency of cancer detection across various organs and tissues[142].

NOVEL CANCER THERAPIES

Tumor malignancy intensifies during extended periods without intervention, posing therapeutic challenges[143]. The current standard of care is unsatisfactory in most cancer types, necessitating the development of novel strategies[144]. Since therapies are more efficient against low-stage tumors, a combination of modern approaches with early detection should significantly improve the quality of life while causing the least side effects[27,145]. Consecutive sections outline novel methods targeting various cancer types and compare their effectiveness and safety to commonly-used therapies. Modern and conventional methods of cancer therapy are depicted in Figure 2.

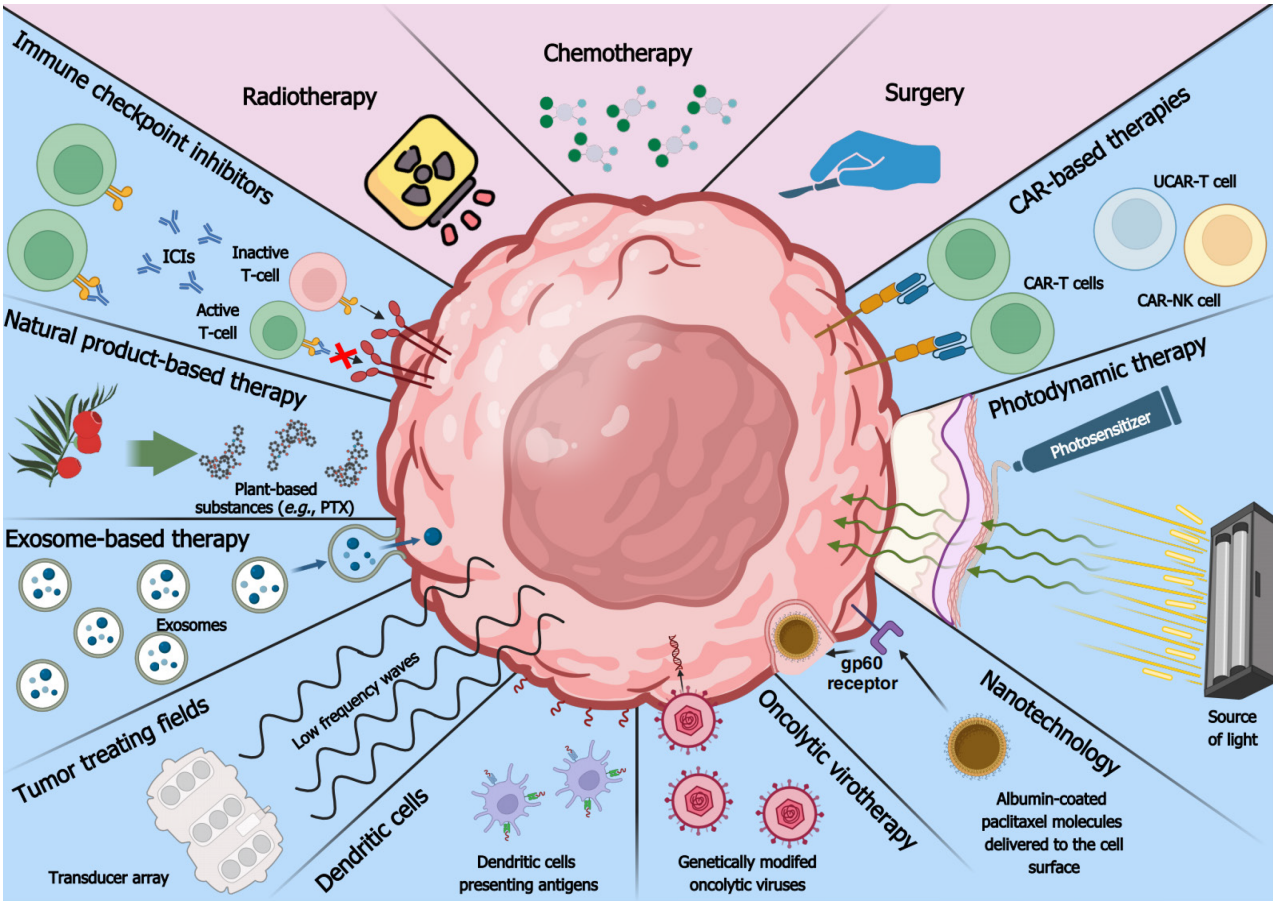

Figure 2 Conventional and modern methods of cancer therapy. Chemotherapy, radiotherapy, and surgery are standard approaches that can be used in combination. Chemotherapeutics are harmful to both cancer cells and normal cells, negatively affecting the quality of life. Radiotherapy counteracts cancer *via* ionizing radiation that deposits high-energy beams in cells. Surgery does not guarantee improvement in the patient's health and carries several risks such as bleeding and damage to surrounding organs and tissues. The current standard of care is unsatisfactory in most cancer types, necessitating the use of novel methods such as oncolytic virotherapy, exosome-based therapy, nanotechnology, dendritic cells, CAR-based therapies, immune checkpoint inhibitors, natural product-based therapy, tumor treating fields, and photodynamic therapy. Figure created with BioRender. CAR: Chimeric antigen receptor; UCAR: Universal CAR; NK: Natural killer; PTX: Paclitaxel.

Oncolytic virotherapy

Oncolytic viruses (OVs) represent an encouraging way of treating cancer since they selectively replicate in tumor cells and lyse them without targeting noncancerous cells. The excessive pool of nucleotides needed to supply rapidly dividing cancer cells creates a favorable environment for the replication of viruses, which are also able to pass the microenvironment[146,147]. OVs manifest their function *via* direct infection and anti-tumor immunity[148]. The former acts through viral replication and host cell death, causing a cascade reaction in which dying cells amplify the presence of OVs within the tumor microenvironment. The second mechanism involves immunogenic cell death, which alarms immune cells by ejecting various particles, such as tumor antigens and damage-associated molecular patterns. Although this process needs further research, it holds promise in counteracting cancer and metastasis[149].

One oncolytic virotherapy, Talimogene laherparepvec, also known as T-Vec or Imlygic, has been approved by the Food and Drug Administration (FDA) and European Medicines Agency for treating melanoma[150]. T-Vec is a modified herpes simplex virus 1, which has been generated from the JS1 viral strand. Modifications include the deletion of the ICP34.5 and ICP47 proteins encoded by *γ34.5* and *α47* genes, as well as the insertion of the human GM-CSF encoded by the *CSF2* gene. Deletion of *γ34.5* limits neurotoxicity and enhances cancer cell-specific viral replication, whereas *α47* deletion enables immune recognition of the injected virus, causing the elimination of the virus outside the tumor microenvironment[151]. The insertion of the human *CSF2* gene promotes dendritic cell (DC) infiltration and maturation, enhancing tumor antigens' presentation to T-cells and improving anti-tumor immunity efficiency[152]. Imlygic is administered as a direct injection into melanoma lesions. A phase III trial showed an overall survival (OS) of 23.3 months, 50% or greater tumor regression in 64% of injected lesions, and mild treatment-associated symptoms in stage IIIB-IV melanoma patients[147]. In comparison, chemotherapy resulted in 5.1-month OS and a partial response rate of 15%-28% [153]. Another limiting factor of chemotherapy was high toxicity associated with severe or life-threatening adverse events, whereas T-Vec caused mild symptoms such as fever or swelling in the injection area[147,153]. Studies are underway into the effects of combining T-Vec with other therapies or its use in other cancer types[150,154].

Another example of an OV-based approach is G47Δ therapy against glioma. G47Δ is a triple mutant of herpes simplex virus 1, which was developed based on the G207 virus. The mutations present in G207 include the deletion of the *γ34.5*

and *α47* genes, as well as the insertion of the *lacZ* gene into *UL39*, *i.e.*, the gene encoding ribonucleotide reductase subunit ICP6, resulting in its inhibition[155,156]. Insertion of the *lacZ* gene limits growth, proliferation, and virulence[157,158]. Oncolytic virus G47Δ can be administered at several sites within the tumor to increase efficiency[156]. The average OS was 20.2 months after G47Δ initiation. Survival at 24 months was 41% (8 out of 19 patients), and at 30 months was 31.58% [156]. Five patients survived more than three years after the onset of therapy, and three patients survived more than eight years. The combination of surgery followed by chemoradiotherapy and adjuvant temozolomide (TMZ) achieved a median OS of 10 months and only 6.7% survival at 24 months[10]. Each patient receiving G47Δ demonstrated only minor side effects (mostly fever in 17 out of 19 patients), and only one patient needed prolonged hospitalization. There were no adverse events that discontinued the therapy. With regard to the standard of care consisting of an invasive operation and chemotherapy, 44% of patients showed systemic adverse events including dizziness, muscle weakness, trouble with memory, and hair loss[159].

Exosome-based therapy

Some cancer therapies end up ineffective due to the inability to infiltrate tumor microenvironment or cancer cells. The value of exosomes in an early diagnosis of oncological patients was described in the section about cancer detection, but their drug delivery characteristics are comparably promising[160]. Compared to other drug delivery methods, exosomes offer greater stability in body fluids, low cytotoxicity or immunogenicity, superior drug protection owing to lipid bilayer structure, the ability to pass the blood-brain barrier, convenient modification for specific tumor types, and the possibility to transport both hydrophobic and hydrophilic molecules[161]. The small size of exosomes alongside their ability to transport molecules of varying sizes and charges make them exquisite candidates for delivering drugs against different types of cancer.

Clinical trials on exosomes in anti-cancer drug delivery are ongoing but a few have yielded preliminary data. The combination of DC-derived exosomes with metronomic cyclophosphamide was tested in a phase II clinical trial on lung cancer. The primary goal of the study was to assess progression-free survival (PFS) in a four-month timeframe[162]. Twenty-two patients were evaluated and their median PFS was 2.2 months. A four-month PFS was achieved in 32% of patients, while OS reached a median value of 15 months. No objective tumor response or cancer-specific T-cell immune response was detected. Although the efficiency results were not exceptional, the treatment was found to be feasible and well tolerated[163]. The safety and tolerability of exosomes were also evaluated in pancreatic cancer and advanced hepatocellular carcinoma. In the case of pancreatic cancer, an ongoing study of KRAS G12D siRNA loaded into exosomes aims to identify maximum tolerated dose and dose-limiting toxicities[164]. The KRAS G12D mutation is the major driver for most pancreatic cancer cases, and its silencing might serve as a potential therapeutic strategy[165]. Although the planned completion date of the study is April 2025, it was already feasible to generate clinical-grade exosomes in mice implanted with patient-derived xenograft harboring KRAS G12D with no severe adverse events[166]. In advanced hepatocellular carcinoma, exoASO-STAT6 is becoming a promising therapy against STAT6, based on targeting with specific anti-sense oligonucleotides[167]. In preclinical *in vitro* trials, exoASO-STAT6 presented selective targeting towards tumor-associated macrophages, which inhibited M2 macrophage polarization and induced an anti-tumor immune response[168]. This could hinder the STAT6 signaling pathway and cancer proliferation and enhance the effectiveness of other methods such as immune checkpoint inhibitors (ICIs) and radiotherapy[169]. Overall, the use of exosomes as drug carriers is encouraging in cancer therapy, but more research is needed to assess their safety and efficiency comprehensively.

DCs

Antigen presentation is enabled by immune components such as macrophages, Langerhans cells, and B-lymphocytes. In addition, a superior approach involves the use of DCs, as they induce adaptive immunity and support the innate immune response against tumors[170,171]. DCs-based therapy utilizes the first and second classes of major histocompatibility complex (MHC) to stimulate the patient's immune system and counteract tumor[172,173]. DCs are primarily involved in determining the compatibility of organs undergoing transplantation but can also be utilized to detect cancer neoantigens. DCs enforce the anti-tumor response, present tumor antigens, and activate T-lymphocytes. The vaccine can be available in an off-the-shelf formulation for repeated long-term administration to a patient[174].

The innovative DC-utilizing therapy termed DCVax-L ensures that the immunosuppressive effects of the tumor are avoided by injecting DCs with cancer-specific antigens extracted from the patient's tumor. This personalized approach allows for a presentation of the tumor antigens and improves the response of the patient's immune system[172,174]. Additionally, the drug can be used in combination with surgical resection of the tumor, chemotherapy, and radiation. Research on DCVax-L is already in the third phase of clinical trials conducted in several countries[174]. The drug was administered intradermally into the patient's arm (with a change of arm for every dose), rendering a less invasive approach than intratumoral injection[174]. The average survival rate among patients with primary glioma was 19.3 months after randomization of the study group, in comparison to 16.5 months for the external control group (ECP) treated with TMZ. Forty-eight months after completion of DCVax-L therapy, the survival rate equaled 15.7% (9.9% in the external control group), whereas 60 months after treatment it was 13% (5.7% in the ECP)[174]. Thus, a more than twofold reduction in the risk of death after completing DC therapy was noted. Among 64 patients with recurrent cancer, a 42% reduction in risk of death was indicated following completion of DCVax-L therapy (13.2 *vs* 7.8 months in ECP). After administering a total of more than 2000 doses of the drug to 64 patients, only five cases of adverse events were detected, with three concerning intracranial edema, one related to severe nausea, and the last regarding lymph node infection. No signs of cytokine storm or autoimmune reaction were detected, and the drug has been proven safe for use[174].

Another example of DC-based therapy is Neo-DCVac, a novel take on counteracting non-small-cell lung cancer (NSCLC). A phase I clinical study on the feasibility and safety was performed among 12 patients who took five doses of the treatment[175]. All patients were in an advanced stage of disease. The treatment showed similar results to chemotherapy, with 7.9 months of OS after Neo-DCVac compared to a median of eight months of OS after chemotherapy [175,176]. The therapy showed synergistic effects with ICIs, only mild-to-moderate adverse events, and no dose-limiting factors. This certifies that DC therapy is a safe treatment option, although the effectiveness of some approaches needs improvement. Further research is required to enhance the Neo-DCVac therapy and determine the optimal dosage.

Chimeric antigen receptor-based therapies

Chimeric antigen receptor T-cell (CAR-T) therapy involves the development of a chimeric antigen receptor that is inserted *via* genetic engineering techniques into T-cells collected from a patient or differentiated from mononuclear blood cells [177,178]. Genetic engineering is typically ensured *via* transfection of the lymphocytes with a lentiviral vector that carries the CAR sequence[178,179]. Following transfection, the chimeric antigen receptor is expressed on the T-lymphocyte surface, which enables precise recognition and elimination of cancer cells. The chimeric antigen receptor is made out of three parts: A signaling domain inside the T-cell, a transmembrane domain, and an antigen recognition domain on the surface of the T-cell[177]. Nowadays, novel CAR-T cells have a modified signaling domain to enhance efficacy or are enriched with an effector memory phenotype[180,181]. Despite showing efficiency in some neoplasms, CAR-T application is challenging in solid tumors and is often correlated with expensive and complicated manufacturing processes, as well as various adverse events such as cytopenia, cytokine release syndrome, and neurotoxicity[181,182].

The first two CAR-T therapies (Kymriah and Yescarta) were approved by the FDA in 2017. Both products were designed to treat B-cell malignancies, namely leukemia and lymphoma, and are targeted to recognize CD19. This antigen is expressed on the surface of B-lymphocytes and their precursors, which guarantees high specificity and limits off-target toxicity[179]. Yescarta showed reliable effectiveness in treating relapsed/refractory large B-cell lymphoma. Survival without the incidence of adverse events, cytokine release syndrome, and neurologic events reached 21.5 months for CAR-T therapy, compared to only 2.5 months for chemotherapy with autologous stem-cell transplantation. In addition, the OS reached 23.9 months for CAR-T *vs* 11.2 months for chemotherapy, with respective response rates of 88% compared to 52% and a complete response rate of 75% compared to only 33%[183]. Unfortunately, 94% of the treated patients experienced severe and life-threatening adverse events, but the toxicity was manageable[183]. Since 2017, various new CAR-T therapies have emerged, and some have already been approved by the FDA, such as Cervykti targeting CD38 in multiple myeloma[184].

To reduce the time and expense involved in classical CAR-T therapies, a new solution has emerged: Universal CAR-T (UCAR-T). Each currently approved CAR-T therapy is made of patient-derived T-lymphocytes to avoid immune rejection. UCAR-T breaks this trend by using allogenic CAR-T cells with T-lymphocytes harvested from healthy donors. This enables the mass production of CAR-T, which decreases the costs and speeds up the time between diagnosis and onset of treatment from two-to-five weeks to only eight days[185,186]. The main issue with UCAR-T is graft *vs* host disease (GvHD), which can manifest when foreign cells are injected into the patient's body, triggering immune rejection [185,187]. Fortunately, this issue can be handled by gene editing, with the most commonly used technique, CRISPR/Cas9, utilized to insert the CAR sequence into a T-cell locus and knockout GvHD-causing genes such as *TRAC*[187]. This increases the safety of UCAR-T and opens avenues for further research on this type of approach. In the future, UCAR-T could substitute current autologous CAR-T therapies, as evidenced by a dual-targeting CD19/CD22 UCAR-T therapy denoted CTA101 that was developed to counteract relapsed/refractory large B-cell lymphoblastic leukemia[186]. CTA101 was tested on six patients in a phase I study; it yielded no GvHD while achieving an 83.3% complete remission rate and a negative result for minimal residual disease after 28 days of treatment[186]. This is higher than treatment *via* Yescarta, which targets CD19 and only obtained a complete response rate of about 75%[183]. Another alternative to CAR-T is CAR-NK, which substitutes the T-lymphocytes with NK cells. Utilizing NK cells reduces toxicity, inflammation, and cytokine release syndrome, as well as improves production efficiency by harvesting cells from different sources or differentiating them from umbilical cord blood[188,189]. Similar to regular CAR-T, NK cells can be transduced with viral vectors and edited by CRISPR/Cas9. A clinical trial featuring CAR-NK cells targeted at CD19-positive lymphoid tumors showed a response rate of 73% and complete remission in 64% of treated patients. No GvHD or cytokine increase has been detected [190]. Novel platforms such as UCAR-T and CAR-NK warrant further investigation in solid tumors.

ICIs

ICIs are biologicals, usually monoclonal antibodies, that prevent the immune-evasive effect of tumors. ICIs enhance the activation, proliferation, and differentiation of T lymphocytes[191,192], improving the patient's immune system as monotherapy or enhancing the activity of another type of treatment. ICIs can bind to various proteins on the cellular surface, but the most common target is the programmed death 1 (PD-1) receptor or its ligand[191]. ICIs attach to the checkpoint protein and block its function. For example, the first ICI approved by the FDA is a human IgG4 monoclonal antibody called nivolumab. It binds to the programmed death 1 receptor on T-cells and prevents its interaction with programmed cell death ligand 1 on tumor cells[191]. This ensures proper lymphocyte T activation, proliferation, and anti-cancer cytokine secretion[193]. In addition to conventional ICIs, it is possible to target phagocytosis checkpoints such as LILRB1/MHC-1 and LILRB2/MHC-1 or NK-cell checkpoints such as KIRs/MHC-1 and PVRIG/CD112[194]; this limits the immunosuppressive effect of tumors and stimulates the elimination of cancer cells *via* phagocytosis or NK-mediated cell death[194].

Not every cancer type is suitable for ICI therapy, *e.g.*, glioma creates a microenvironment unfavorable for binding antigens to the cancer cell surface. However, ICIs hold promise in lung cancer. It was found that a frequently used ICI known as durvalumab increased OS to 12.9 months when combined with chemotherapy in comparison to chemotherapy

alone which reached 10.5 months[195]. Although ICIs can be effective, new inhibitors should be developed to improve clinical endpoints further. One of the newer antibodies targeting PD-1 is serplulimab. This humanized IgG4 monoclonal antibody was tested in a phase III randomized clinical trial on extensive-stage small-cell lung cancer, where each patient received the treatment intravenously every three weeks[196]. Serplulimab improved OS to a greater extent than durvalumab: The median OS of patients receiving serplulimab combined with chemotherapy reached 15.4 months compared to 10.9 months for individuals receiving chemotherapy. However, ICI also caused severe or life-threatening adverse events in 33.2% of patients[197]. Although ICIs show promising results, further development is needed to enhance their efficacy, limit adverse effects, and apply them to a broader range of cancer types. A deeper understanding of immune checkpoints on cancer cells and discovering novel targets or inhibitors will facilitate progress in ICI therapy.

Natural product-based therapy

The search for anti-cancer compounds has expanded to include substances found in natural sources and the everyday diet. Plant-based substances are of particular interest, despite sometimes providing only small amount of compound. Nowadays, it is possible to synthesize them chemically in a laboratory. A prime example is the natural substance paclitaxel (PTX), a strong anti-cancer drug that laid the foundations for new therapies against many malignancies such as ovarian, colorectal, bladder, pancreatic, lung, and squamous cell carcinoma[198-201]. PTX was originally produced from yew tree bark but is now manufactured from precursors such as 10-deacetylbaccatin III[202]. PTX belongs to the group of anti-cancer drugs so-called "mitotic poisons" that bind tubulin. The compound prevents microtubule depolymerization, impairs the function of the mitotic spindle, stops cell cycle progression, and ultimately inhibits cancer cell proliferation [199,200]. While mitotic arrest is the primary mechanism, PTX also induces p53-mediated apoptosis. In some cancer cells, PTX has been connected to elevated levels of reactive oxygen species (ROS) and upregulated genes associated with endoplasmic reticulum stress. It remains to be determined whether the endoplasmic reticulum stress derives from gene dysregulation caused by p53 activation[203].

A phase III clinical trial examined the use of PTX in combination with carboplatin for the treatment of ovarian cancer. This combination was compared to the conventional PTX-doxorubicin-cisplatin regimen. Although PTX-carboplatin treatment resulted in lower OS (37 *vs* 41 months), it was a preferred choice due to the improved quality of life and reduced toxicity for patients. This therapy was also recommended as a first-line approach against advanced endometrial cancer[204,205]. Another trial with 861 metastatic pancreatic cancer patients found that gemcitabine plus albumin-bound PTX was superior to gemcitabine alone. Improvements in response rates (23% *vs* 7%), PFS (5.5 *vs* 3.7 months), and OS (8.5 *vs* 6.7 months) were noted in the combination therapy. The nab-PTX-gemcitabine group showed higher one-year survival (35% *vs* 22%) and two-year survival (9% *vs* 4%) compared to the gemcitabine group[206]. The distinct mechanism of action of PTX highlights its potential to enhance patient outcomes.

Nanotechnology

Nanotechnology is a wide scientific branch utilizing submicrometer-sized materials. It can be used for detecting and marking tumors *via* different fluorescent or light-emitting probes[207]. Nanotechnological vesicles with a size of < 100 nm are being used for drug and probe delivery to tumor cells. The process includes the formation of the nanostructure, surface modification of the nanocarrier (to target cancer cells), transportation of the nanocarrier, and release of the drug in the target site or binding with the attached probe[208]. For safe and effective therapy, it is necessary to target cancer cells specifically. Fortunately, tumors are characterized by elevated vascular permeability and decreased lymphatic drainage, allowing passive targeting[209]. In contrast, active targeting involves the interaction between targets and ligands; the latter are located on nanoparticles and specifically recognize targets overexpressed in cancer cells (peptides, amino acids, carbohydrates, antibodies)[210]. This results in endocytosis of the nanoparticle together with its payload, *e.g.*, proteins or siRNA[211]. Nanotechnological vesicles come in various forms, such as polymeric or nonpolymeric nanoparticles, quantum specks, nanotubules, dendrimers, and lipid- or micelle-based nanoparticles.

Nanoparticle albumin-bound paclitaxel (nab-PTX), a novel form of the well-known agent, has demonstrated good effectiveness and safety. Nanoparticles enable the delivery of higher PTX levels in shorter infusion times compared to the commonly used solvent-based formulation[212]. Regarding stomach, breast, and lung tumors, the effectiveness of nab-PTX was found to be comparably effective as a standard of care[213-215]; however, it turned out to be of greater efficacy against NSCLC than docetaxel[216]. A phase III multicenter, randomized clinical trial investigated the efficiency of nab-PTX against NSCLC, presenting longer survival of patients treated with nab-PTX than those treated with docetaxel: OS of 16.2 months compared to 13.6 months and PFS of 4.2 months *vs* 3.4 months. Another significant difference was the objective response rate, which reached 29.9% for nanoparticle therapy compared to 15.4% for docetaxel[212]. This suggests that nanotechnology can be superior to standard treatment and perhaps will facilitate the development of future anti-cancer approaches.

Tumor-treating fields

Another method employs tumor-treating fields (TTFs), in which alternating, low-intensity, intermediate-frequency electric fields are transmitted transdermally to the tumor site; they selectively affect dividing cancer cells with minimal effect on non-extensively proliferating normal cells. The frequency of the alternating current can range from 100 to 400 kHz but is typically 150 kHz for NSCLC and pleural mesothelioma or 200 kHz for glioblastoma[159,217,218]. TTFs are approved by the FDA for the last two mentioned tumors, and are expected to receive approval soon for NSCLC[219-221]. Other frequencies are being evaluated to suit other cancer types. TTFs are applied by using special transducer arrays with electrodes that are activated sequentially to cause a change in the direction of the current[217]. The direction of electric charge flow and electrode placement can be determined using special software such as NovoTAL[159]. At a certain

frequency, such alternating fields affect cellular structures. TTFs can be delivered to various parts of the human body, such as the patient's scalp for glioma treatment or the thoracic region for NSCLC treatment. Electric fields can be applied for several hours a day (most often over 18 h daily) without significant harm to healthy normal cells but causing various effects on tumor cells[159,218]. TTFs cause an aberrant mitotic exit, prevent mitotic spindle assembly, perturb mitotic tubulin, and adjust cell membrane potential to increase Ca^{2+} influx and reduce microtubule polymerization[159,222]. They can also increase blood-brain barrier permeability, decrease cell dispersion, reverse markers of epithelial-to-mesenchymal transition, as well as inhibit cancer cell proliferation and metastasis[217]. Moreover, TTFs can induce autophagy, replication stress, MHC class II expression, macrophage differentiation, and various immunological effects such as immunogenic cell death[223,224]. This multimodality leads to substantial efficiency that is combined with great safety.

TTFs have been proven effective in targeting glioma, pleural mesothelioma, and NSCLC. For the latter, standard therapy (docetaxel and nivolumab, pembrolizumab, or atezolizumab) combined with TTFs achieved a median OS of 13.2 months compared to 9.9 months for standard treatment alone. Related adverse events were reported in 95 out of 133 patients; 81 patients had mild-to-moderate disorders of the skin and subcutaneous tissue[218]. In glioma, one of the clinical trials reported an OS of 27.2 months in newly diagnosed glioma patients receiving TTF with TMZ compared to 15.2 months in those with TMZ only[225]. Another study showed an OS of 20.9 months in the TTFs + TMZ group compared to 16 months in the TMZ group with no serious adverse events: Only mild-to-moderate skin toxicity underneath transducer arrays[159]. A phase II study on pleural mesothelioma showed a median OS of 18.2 months compared to 14.2 months in the control group and predominantly mild-to-moderate adverse events[220]. These results hold promise for future research on the efficiency of TTFs and their use in various cancer types.

Photodynamic therapy

Photodynamic therapy (PDT) is an approach targeting lesions that can be pre-cancerous such as actinic keratosis, or cancerous such as mycosis fungoides and basal cell carcinoma[226,227]. Despite being predominantly investigated in skin lesions, there is ongoing research on PDT in glioma, leukemia, and tumors of the bladder, colon, breast, and cervix[228,229]. Effective therapy requires three essential components, namely a photosensitizer (PS), light, and oxygen[230]. The PS is often an ointment or cream accumulating in pathological tissues[231]. It is designed to absorb light of a given wavelength to minimize unwanted activation after sunlight exposure. The PS only manifests its toxicity in the region of application, improving precision and safety[230]. The most commonly tested PS is hypericin[227,228]. After light absorption, the PS may transition to an excited singlet state and emit fluorescence as it returns to the ground state. The singlet state can also convert to a triplet state that interacts with endogenous substances to produce free radicals such as H_2O_2 and O_2^-. Alternatively, the triplet state can react with molecular oxygen to form $^1O2$; this is the most common scenario for ROS generation by PS in PDT[232-234]. Singlet oxygen generation leads to significant toxicity, signaling pathway alterations, apoptosis, and necrosis in PS-absorbing areas[228,230].

Clinical trials have shown that hypericin-associated PDT can effectively treat mycosis fungoides, a type of cutaneous T-cell lymphoma[227,228]. A phase III, double-blind, randomized clinical trial featuring 169 patients utilized hypericin as a PS, a light panel providing a 500-650 nm spectrum, and oxygen to enable ROS generation[227]. The results indicated that hypericin offers better efficiency than a placebo comparator, which was chosen due to the lack of FDA-approved standard skin-directed therapy for mycosis fungoides. The primary outcome was index lesion response rate (ILRR) measured after each of the three treatment cycles, the third of which was optional. The hypericin group demonstrated better ILRR than the placebo after the first cycle (16% *vs* 4%) and similar after the second cycle (40% *vs* 22%). In the third cycle, ILRR increased to 49% for patients who participated in all three cycles, but no data from the comparative placebo group was provided. Both hypericin and placebo achieved similar safety profiles but slightly in favor of the placebo ointment. The most common adverse events were subcutaneous tissue-related disorders (13.5%-17.3% across treatment cycles *vs* 10.5% for placebo) and application-site reactions (3.2%-6.9% across treatment cycles *vs* 4% for placebo). Since there were no serious adverse events related to PDT, further research is advisable to develop new PSs and enhance their universal application and effectiveness.

CONCLUSION

The continuously increasing incidence of cancer cases and the related mortality index emphasize the need for more precise detection and therapeutic approaches. Conventional methods, although still common in clinical practice, pose many limitations which justify the development of novel strategies for earlier diagnosis or more reliable and precise targeting of cancer. The present paper integrates available data on conventional and modern methods of cancer detection and therapy to facilitate an understanding of the rapidly advancing field and its future directions. Modern techniques have their drawbacks, and there is still a need to develop new detection strategies and therapeutic approaches to improve sensitivity, specificity, safety, and efficacy. Strategies utilizing DCs, nanotechnology, and plant-based agents are promising, but their effectiveness depends on the specific therapy, with some being superior or comparable to the standard of care. Oncolytic virotherapy greatly improves survival and entails only minor side effects, but multiple administrations may raise safety concerns. PDT or an exosome-based approach requires more data to understand related outcomes. ICIs and classical chimeric antigen receptor-based therapies pose many adverse effects, emphasizing the need for improved solutions. TTFs predominantly entail mild adverse events, and their ability to improve survival is encouraging. All things considered, the approval of some modern methods by the FDA emphasizes that efforts should continue.

FOOTNOTES

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Country of origin: Poland

ORCID number: Piotr Gromek 0009-0005-8093-8481; Zuzanna Senkowska 0009-0006-1243-721X; Elżbieta Płuciennik 0000-0002-6682-0402; Zbigniew Pasieka 0000-0001-8931-0213; Lin-Yong Zhao 0000-0003-0884-4657; Adrianna Gielecińska 0000-0001-9562-3582; Mateusz Kciuk 0000-0002-8616-3825; Karol Kłosiński 0000-0003-0962-228X; Żaneta Kałuzińska-Kołat 0000-0002-2335-3293; Damian Kołat 0000-0002-1086-3796.

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Non-alcoholic fatty liver disease in type 2 diabetes: Emerging evidence of benefit of peroxisome proliferator-activated receptors agonists and incretin-based therapies

Subhodip Pramanik, Partha Pal, Sayantan Ray

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Subhodip Pramanik, Department of Endocrinology, Neotia Getwel Multispecialty Hospital, Siliguri 734010, West Bengal, India

Partha Pal, Department of Medical Gastroenterology, Asian Institute of Gastroenterology, Hyderabad 500082, India

Sayantan Ray, Department of Endocrinology, All India Institute of Medical Sciences, Bhubaneswar, Bhubaneswar 751019, Odisha, India

Corresponding author: Sayantan Ray, MBBS, MD, Assistant Professor, Department of Endocrinology, All India Institute of Medical Sciences, Bhubaneswar, Sijua, Patrapada, Bhubaneswar 751019, Odisha, India. sayantan.ray30@gmail.com

Abstract

Nonalcoholic fatty liver disease (NAFLD) is a global epidemic, affecting more than half of the people living with type 2 diabetes (T2D). The relationship between NAFLD and T2D is bidirectional and the presence of one perpetuates the other, which significantly increases the hepatic as well as extrahepatic complications. Until recently, there was no approved pharmacological treatment for NAFLD/ nonalcoholic steatohepatitits (NASH). However, there is evidence that drugs used for diabetes may have beneficial effects on NAFLD. Insulin sensitizers acting through peroxisome proliferator-activated receptor (PPAR) modulation act on multiple levels of NAFLD pathogenesis. Pioglitazone (PPARγ agonist) and saroglitazar (PPARα/γ agonist) are particularly beneficial and recommended by several authoritative bodies for treating NAFLD in T2D, although data on biopsy-proven NASH are lacking with the latter. Initial data on elafibanor (PPAR α/δ agonist) and Lanifibranor (pan PPAR agonist) are promising. On the other hand, incretin therapies based on glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RA) and dual- and triple-hormone receptor co-agonists reported impressive weight loss and may have anti-inflammatory and antifibrotic properties. GLP-1 RAs have shown beneficial effects on NAFLD/NASH and more studies on potential direct effects on liver function by dual- and triple-agonists are required. Furthermore, the long-term safety of these therapies in NAFLD needs to be established. Collaborative efforts among healthcare providers such as primary care doctors, hepatologists, and endocrinologists are warranted for selecting patients for the best possible management of NAFLD in T2D.

Key Words: Non-alcoholic fatty liver disease; Type 2 diabetes; Evidence; PPAR agonists; Incretin-based therapies

Core Tip: Co-existence of non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM) synergistically act to increase the risk of adverse hepatic and extra hepatic outcomes. T2DM is an established risk factor for NAFLD progression to NASH, advanced fibrosis or cirrhosis. Timely intervention in these populations could have a significant effect on liver- related outcomes. Newer dual and pan-PPAR agonists show promising results in patients with NAFLD/NASH and T2DM. Incretin-based therapy for the treatment of NAFLD is currently being explored. With better understanding of the complex interaction between T2DM and NAFLD, PPAR agonists and incretin-based therapies are likely to provide more effective approach of NAFLD management in T2DM.

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INTRODUCTION

The coexistence of non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes (T2D) synergistically act to increase the risk of adverse hepatic and extrahepatic outcomes. The global prevalence of NAFLD in people with T2D has been estimated at nearly 56% in a large meta-analysis of observational studies. More than one-third of people with T2D have nonalcoholic steatohepatitis (NASH), and around 1 in 6 carries advanced fibrosis[1]. T2D is an established risk factor for NAFLD progression to NASH, advanced fibrosis, or cirrhosis. Considering the prospective clinical implications of T2D in NAFLD, timely intervention in these patients could have a significant effect on liver-related outcomes. NAFLD and T2D share underlying altered pathophysiological mechanisms, including insulin resistance (IR). Expectedly, some anti-diabetic drug classes might exert favorable effects on NAFLD, with prevention of adverse cardiovascular (CV) events as well[2]. However, an unmet need for more focused research is there on how glycemic control through insulin sensitizers or incretin-based therapies might halt or revert the progression of NAFLD in individuals with T2D. The present mini-review summarizes the evidence on strong and significant association between T2D and the risk of adverse clinical outcomes in NAFLD, the therapeutic potential of peroxisome proliferator-activated receptors (PPAR) agonists for NASH and NASH-related hepatic morbidity, and lastly the key scientific advances regarding the use of incretins for treating NAFLD.

NAFLD AND T2D- THE COMPLEX LINK

From diabetes to NAFLD/NASH

The association between T2D and NAFLD is more complex than earlier thought and the relationship appears to be bidirectional[3]. The underlying pathophysiological mechanisms for the development of NAFLD are primarily the glucose and lipid metabolism alterations, with IR, elucidating the close link between T2D and NAFLD. Increased adiposity, often found in NAFLD and T2D, is coupled with adipocyte IR and dysfunction. Dysfunctional adipose tissue (AT) exhibits resistance to the antilipolytic effect of insulin. The consequent enhanced lipolysis and release of free fatty acids (FFAs) and glycerol are responsible for the accumulation of triglyceride and lipotoxicity in the liver, and other organs. Overflow of FAs to the liver also results in increased cellular levels of toxic lipids such as diacylglycerols, ceramides, and long-chain fatty acyl-coenzyme A (CoA), which are involved in inflammatory pathways (Figure 1)[4]. Excess FFAs also induce mitochondrial dysfunction, an increase in oxidative stress, and uncoupled oxidative phosphorylation. A fibrogenic response in hepatic stellate cells may be activated that can stimulate the progression to NASH and cirrhosis, and reactive oxygen species production (Figure 1)[5]. Dysfunction of AT and IR leads to the enhanced release of pro-inflammatory cytokines and decreased release of anti-inflammatory adipokines by AT. These cytokines can damage the liver directly, or indirectly, by raising oxidative stress, liver fibrosis, and the development of tumors[6,7]. Being closely interrelated, both glucotoxicity and lipotoxicity contribute to worsening IR and impaired insulin secretion (Figure 1)[8]. Hyperglycemia induces hepatic de-novo lipogenesis (DNL). Saturated fatty acid palmitate, the primary product of lipogenesis can induce hepatic inflammation and endoplasmic reticulum stress. Persistently elevated blood glucose levels promote DNL through direct and indirect mechanisms: the direct mechanism is by enhancing the activity of TCA cycle and Acyl CoA synthesis that acts as a substrate of both DNL and gluconeogenesis, and the indirect mechanism is through the transcription factor carbohydrate response element binding protein and liver X receptor αwhich sequentially promote unfavorable gene transcriptions.

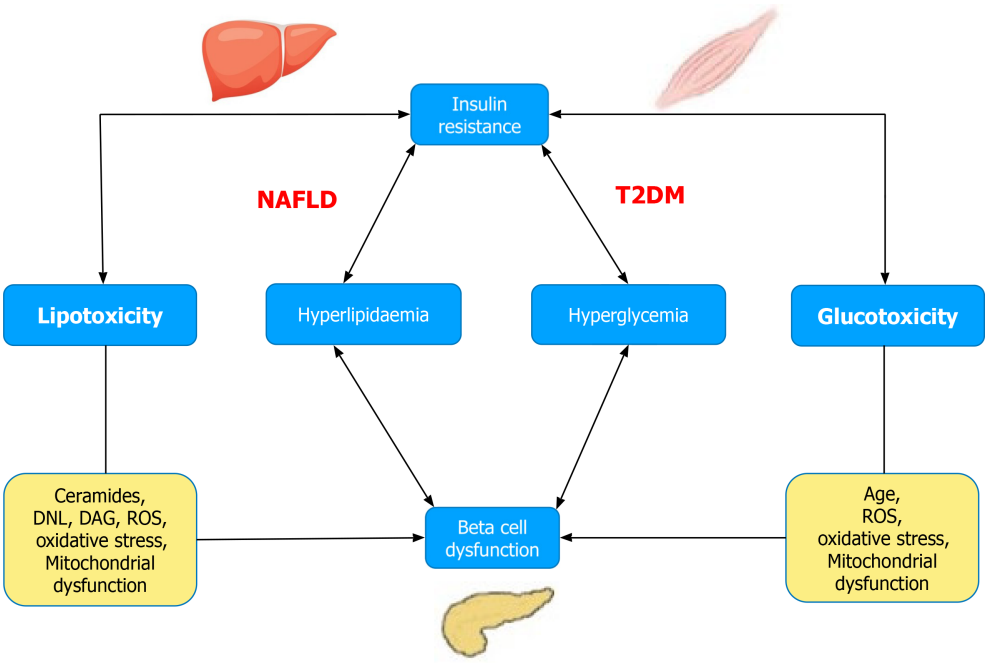


Figure 1 Relationship between lipo- and glucotoxicity, insulin resistance, and beta-cell function. DAG: Diacylglycerol; DNL: *De novo* lipogenesis; NAFLD: Non-alcoholic fatty liver disease; ROS: Reactive oxygen species; T2D: Type 2 diabetes.

Progression of NAFLD in T2D

T2D is one of the strongest risk factors for the progression of NAFLD to NASH, cirrhosis, or hepatocellular carcinoma (HCC). An unhealthy lifestyle, *via* the promotion of gut dysbiosis and T2D-associated hyperglycemia and hyperinsulinemia, influences the transition from isolated steatosis to NASH and the progression of NASH. T2D-associated hyperglycemia also promotes the progression from NASH to hepatic fibrosis and HCC (Figure 2)[8]. Swedish National Diabetes Registry showed that severe liver disease risk is higher in T2D patients than in the general population. In people with T2D, risk factors associated with severe liver disease were older age, male gender, higher body mass index, smoking, hypertension, and microalbuminuria[9]. Among persons with NAFLD, those with T2D are at greater risk of developing advanced stages of fibrosis and subsequently hepatic decompensation, HCC, and liver-related mortality than those without T2D[10,11]. Huang *et al*[12] report the findings of a recent large meta-analysis comprising 2016 ethnically diverse participants. Compared with individuals without diabetes, participants with T2D had an elevated risk of hepatic decompensation and HCC despite adjustment for multiple factors having a known association with the progression of NAFLD. In general, liver fibrosis progresses by one stage over 7 years for people with NASH, but data are limited regarding the time to fibrosis progression in people with T2D compared with people without T2D[13]. In a recent comprehensive, multicentric study of patients with paired liver biopsies from the Nonalcoholic Steatohepatitis Clinical Research Network, the authors found that the cumulative incidence of fibrosis progression was considerably higher in participants with T2D compared with individuals without T2D. T2D remained an important predictor of the progression of fibrosis, even after controlling for several potential confounders[14]. Altogether, these findings provide convincing clinical data to point out that T2D is associated with advanced-stage liver disease, faster disease progression, and worse hepatic outcomes in people with NAFLD. Consequently, it is compulsory to screen for the presence and severity of NAFLD in all adults with T2D with liver enzymes in a wider range than usually considered[15]. Poor glycemic control has been linked to severe long-term complications, including advanced liver disease, and adverse outcomes in NAFLD; however, an ideal HbA1c cutoff value to detect people at risk of those outcomes is yet to be identified.

THERAPEUTIC ROLE OF PPAR AGONISTS

Rationale

PPARs are nuclear receptors for specific endogenous ligands and act as transcription factors involved in the regulation of lipid metabolism, glucose homeostasis, and inflammation[16]. Endogenous ligands include free fatty acids (FAs), eicosanoids, and various complex lipids. PPAR forms a heterodimer with retinoid X receptor once ligands bind to it, and the complex binds to the response element that regulates the gene expression which is important for fatty acid metabolism[17]. There are three PPAR isoforms, PPAR α is largely present in the liver involved in fatty acid oxidation, and exerts anti-inflammatory action through complex regulation of nuclear factor kappa-light-chain-enhancer of activated B cells[18]. PPAR β/δ is expressed mainly in skeletal muscle but is also present in adipose tissue and skin and has a significant role in controlling lipid metabolism[19]. PPAR γ is highly expressed in adipose tissue and plays an important role in the regulation of adipogenesis, adipocyte differentiation, and fatty acid metabolism[20]. Together,

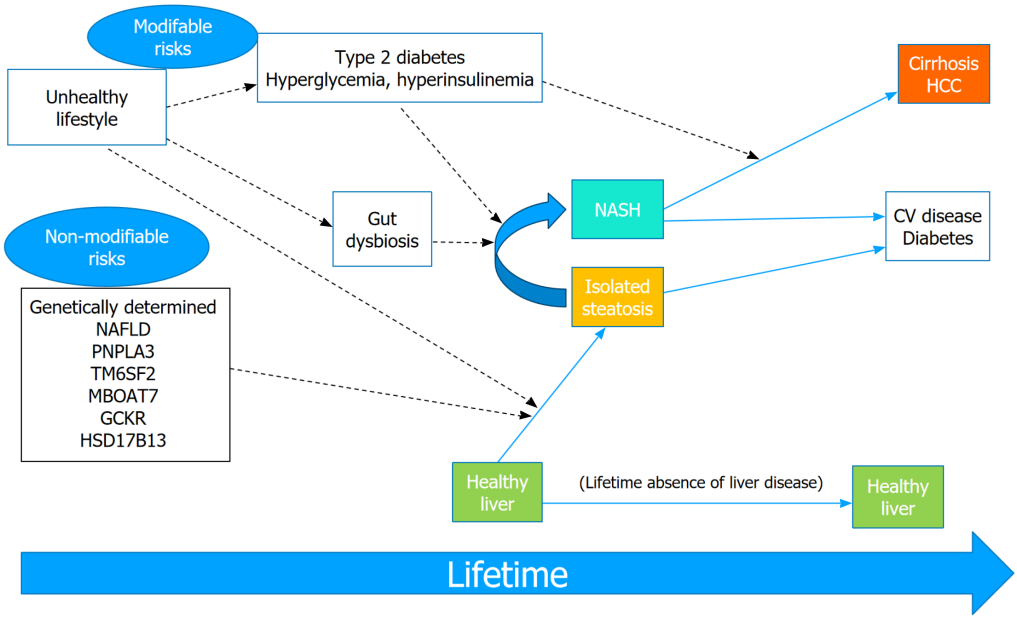


Figure 2 Modifiable and non-modifiable risk factors of non-alcoholic fatty liver disease and its progression to severe liver disease. Dashed arrows indicate factors that promote or predispose to disease. Among non-modifiable risk factors, most important genetic variants of nonalcoholic fatty liver disease are being extensively studied. Type 2 diabetes-associated hyperglycemia induces progression from nonalcoholic steatohepatitis to hepatic fibrosis and HCC. CV: Cardiovascular; HCC: Hepatocellular carcinoma; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

PPAR agonists have a role in insulin sensitivity, inflammation, and dyslipidemia and thus act on multiple levels in NAFLD pathogenesis. This makes it an attractive option for drug development.

PPAR single agonists

Given the differential actions of different PPAR agonists, there has been a significant effort in the development of agonists for single receptors. They have been tried in different conditions including NAFLD, the results of which are encouraging. The agents, and trials with biochemical and histological outcomes of single agonists are summarized in Table 1[21-27].

PPARα agonists: Fibrates are considered as PPARα agonists, albeit weak, and have limited clinical efficacy in NAFLD. In a study by Fernández-Miranda *et al*[21] 16 patients with NAFLD were treated with fenofibrate 200 mg/d for 48 wk and found a significant reduction in triglyceride and other liver enzymes. However, there was no significant improvement in histologic parameters. Another fibrate, clofibrate was compared with ursodeoxycholic acid in 40 patients for 1 year but no advantage was found in the clofibrate group. Newer selective PPARα-specific agonists, known as selective PPAR modulators, are in different phases of development[28,29].

PPAR β/δ agonists: Data is sparse with these PPAR isotypes. Two agents GW501516 and MBX-8025 have been tried in short trials[24,25]. They have shown some benefit in metabolic parameters (Table 1) but no histologic data is available. Further studies are required to establish any role of these agents in NAFLD.

PPARγ agonists: Thiazolidinediones are strong PPARγ agonists widely investigated in NAFLD. They are insulin-sensitizing agents and have a significant role in lipid metabolism. Rosiglitazone was compared with placebo for 1-year therapy in FLIRT trial[25] and found to have improved steatosis (47% *vs* 16%) and transaminitis (38% *vs* 7%), but lacked improvement in fibrosis and NAFLD activity score. Weight gain and painful swollen legs were significant side effects in the rosiglitazone arm. In the PIVENS trial[27], Pioglitazone was compared with vitamin E and placebo for 96 wk in patients with NASH where pioglitazone demonstrated a reduction in hepatic steatosis, lobular inflammation, and hepatic enzymes. A meta-analysis including 3 high-quality randomized controlled trials (RCTs) comparing pioglitazone *vs* placebo in NAFLD showed significant improvement in liver fibrosis[30]. Currently, pioglitazone is recommended in persons with T2D and biopsy-proven NASH by the American Association of Clinical Endocrinologists (AACE)[31] and recommended in both diabetic and non-diabetic adult patients with or without fibrosis by the Indian National Association for the study of the liver (INASL)[32] (Strong recommendation, high strength of evidence). However, the use of PPAR agonists can be associated with poor bone quality (in post-menopausal females) and dose-dependent pedal edema and weight gain.

Dual and pan PPAR agonists

Combination therapy using dual or pan agonists is an attractive choice as the most beneficial effects on NAFLD can be seen with the least side effects. For example, they can produce an antihyperlipidemic (PPARα) effect with insulin sensitization (PPARγ) and increase β-oxidation in the liver and skeletal muscle (α and β/δ). The efficacy can also be improved by synergistic action of different isoforms on fatty acid metabolism and insulin sensitivity. On the other hand, the side

Table 1 Summary of studies of peroxisome proliferator-activated receptors single agonists in nonalcoholic fatty liver disease

Drug class and mechanism	Agent	Trial design	Biochemical response	Histological response	Comments
PPARα (Fatty acid oxidation and Anti-inflammatory)[21,22]	Fenofibrate	16 patients, 48 wk *vs* placebo	Significant reduction in triglyceride and liver enzymes	Decreased ballooning, grade steatosis, inflammation/fibrosis no change	Limited efficacy
	Clofibrate	40 patients 1 yr *vs* UDCA	Reduced ALT	No change	
PPAR β/δ (glucose homeostasis and insulin sensitivity)[23,24]	GW501516	6 patients, 2 wk *vs* placebo	Reduced TG and LDL	No data	Abandoned due to cancer risk in preclinical studies
	MBX-8025	181 overweight patients, 2 wk *vs* placebo	Favorable lipid profile and decreased liver enzymes	No data	Need more data
PPARγ (adipogenesis, insulin sensitization, fatty acid oxidation)[25-27]	Rosiglitazone	63 patients, 52 wk *vs* placebo	Normalized transaminase levels (38% *vs* 7%, $P = 0.005$)	Improved steatosis (47% *vs* 16%; $P = 0.014$), although only half of the patients responded, no change of other histologic parameters	Weight gain and painful swollen legs in rosiglitazone arm
	Pioglitazone	RCT, 61 patients, 12 months placebo or pioglitazone had paired biopsies	Improvement of ALT and GGT	Hepatocellular injury ($P = 0.005$), Mallory–Denk bodies ($P = 0.004$), and fibrosis ($P = 0.05$) were reduced in patients treated with pioglitazone	Weight gain with pioglitazone
		RCT, 259 patients pioglitazone *vs* vitamin E *vs* placebo 96 wk	Improvement of transaminases in the vitamin E and pioglitazone arm	Improvement in NASH as compared with placebo (pioglitazone $P = 0.04$), significant reductions in steatosis, lobular inflammation and fibrosis in pioglitazone arm	Weight gain in pioglitazone group

ALT: Alanine aminotransferase, GGT: Gamma glutamyl transferase, LDL: Low density lipoprotein, PPAR: Peroxisome proliferator-activated receptor, RCT: Randomized controlled trial, TG: Triglyceride, UDCA: Ursodeoxycolic acid.

effects seen with the individual class of drugs like heart failure or weight gain with pioglitazone can be minimized. The agents and trials with biochemical and histological outcomes of dual/pan agonists are summarized in Table 2[33-40].

PPAR α/δ agonist: Elafibranor, an agonist of PPARα and PPARδ, works on insulin sensitivity, glucose homeostasis, and lipid metabolism. It has been studied in an RCT by Ratziu *et al*[33] (Golden trial) where two doses (80 mg and 120 mg) of Elafibranor were compared with a placebo. In the intention-to-treat analysis, there was no improvement in the resolution of NASH without worsening fibrosis (primary outcome). However, Elafibranor 120 mg became superior to placebo in 19% *vs* 12% in the placebo group ($P = 0.045$), based on a post hoc analysis for the modified definition of NASH resolution after excluding patients with mild steatohepatitis from the analysis. Another study RESOLVE IT (NCT02704403) was terminated early due to the limited effect of Elafibranor on surrogate efficacy endpoints. No safety signal was found in the studies.

PPAR α/γ agonists: Glitazars are PPAR α/γ agonists and improve dyslipidemia through action and insulin sensitivity through g action thus addressing two important issues of NAFLD. Initial glitazars, Tesaglitazar, Muraglitazar, and Aleglitazar, although showed significant improvement in dyslipidemia, trials for NFALD were terminated early due to nephrotoxicity, cardiotoxicity, and gastrointestinal hemorrhage respectively[34-36]. Saroglitazar is the new molecule in this class containing a unique pyrol moiety and lacks the glitazone ring and unlike its congeners has predominant PPARα action and moderate PPARγ action. Thus, it lacks the typical glitazone side effects. In mice models, saroglitazar has been shown to reduce histological NASH as compared with pioglitazone and fenofibrate[41]. Serum transaminase levels improved in patients with T2D treated with saroglitazar as compared with placebo[38,42]. A similar trial is ongoing at the United Nations with saroglitazar (NCT03061721) which will evaluate the improvement of serum transaminases and hepatic steatosis on MR imaging-estimated proton density fat fraction (MR-PDFF). Additionally, a recent phase III study (CTRI/2015/10/006236) in India is currently evaluating the histological efficacy of saroglitazar in comparison with a placebo in patients with biopsy-proven NASH.

Pan PPAR agonist: Pan PPAR agonists are still in various phases of development and many animal data are published. Bezafibrate and Lanifibranor have shown anti-lipid, anti-inflammatory, and antifibrotic properties in rats. An ongoing study is evaluating the efficacy of lanifibranor in patients with diabetes and NAFLD (NCT03459079). These agents may have a promising role in the management of NAFLD.

To conclude, given the multiple actions of the PPAR on lipid metabolism, oxidation of FAs, glucose homeostasis, and inflammation, PPAR agonists are attractive targets for the treatment of patients with NAFLD. Of all the PPAR agonists, the PPARα agonist pioglitazone is the most extensively evaluated and barring a few side effects, is most useful in patients with NAFLD and recommended by AACE and INASL guidelines. Emerging data of dual PPAR agonists and pan-PPAR

Table 2 Summary of studies of peroxisome proliferator-activated receptors dual and pan agonists in nonalcoholic fatty liver disease

Drug class and mechanism	Agent	Trial design	Biochemical response	Histological response	Comments
PPAR α/δ agonist[33]	Elafibranor	276 patients, Elafibranor 80 mg *vs* 120 mg *vs* placebo, 52 wk, GOLDEN trial	Liver enzymes, lipids, glucose profiles, and markers of systemic inflammation were significantly reduced in the elafibranor 120-mg group	Elafibranor 120 mg was superior to the placebo, with NASH resolution without worsening of fibrosis in 19% versus 12% in the placebo group (P = 0.045), based on a post hoc analysis for the modified definition	No change in primary outcome in intention to treat analysis
		1070 patients, Elafibranor 120 mg *vs* placebo, 72 wk, RESOLVE IT trial	Improvement in TG, ALT, and GGT	138 (19.2%) patients in the Ela group and 52 (14.7%) patients in the placebo group achieved resolution of NASH without worsening of fibrosis (P = 0.066)	Despite the absence of safety signals, the RESOLVE-IT trial was discontinued due to the limited effect of Ela on surrogate efficacy endpoints
PPAR α/γ agonist[34-38]	Tesaglitazar, Muraglitazar, Aleglitazar		Favorable lipid profile with muraglitazar	No change	Trials terminated early due to nephrotoxicity, cardiotoxicity and gastrointestinal hemorrhage respectively
	Saroglitazar	106 patients, 16 wk *vs* placebo	Improvement in ALT and lipid profile	No data	DCGI approved for NAFLD in India
		85 patients, 12 wk *vs* placebo	Improvement in ALT and TG	Significant reduction in liver fibrosis (fibroscan)	
Pan PPAR agonists[39,40]	Bezafibrate		Improve HbA1c and atherogenic dyslipidemia in mice	No data	Mostly animal data. Human studies are ongoing
	Lanifibranor		Improve insulin sensitivity in mice	Improve steatosis and fibrosis in liver tissue in mice	

ALT: Alanine aminotransferase; DCGI: Drug Controller General of India; GGT: Gamma glutamyl transferase; PPAR: Peroxisome proliferator-activated receptor; TG: Triglyceride.

agonists appear encouraging and may hold promise for patients with NAFLD.

THERAPEUTIC ROLE OF INCRETIN-BASED THERAPIES

In response to the intake of food, incretins play an important role in the regulation of blood glucose levels by enhancing insulin secretion from β-cells of the pancreas after meals. There are two main incretin hormones which are glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). GLP-1 is produced by L cells in the ileum and colon whereas GIP is produced from K cells in the duodenum and jejunum. The release of both is triggered as a response to the presence of nutrients (carbohydrates for both GLP1 and GIP, fat for GIP) in the digestive system. GLP-1 and GIP enhance glucose-dependent insulin release and inhibit glucagon secretion (glucagon stimulates the liver to release glucose) into the bloodstream. In addition, GLP-1 delays gastric emptying, regulates nutrient absorption, and promotes satiety whereas GIP promotes fat storage in adipose tissue[43].

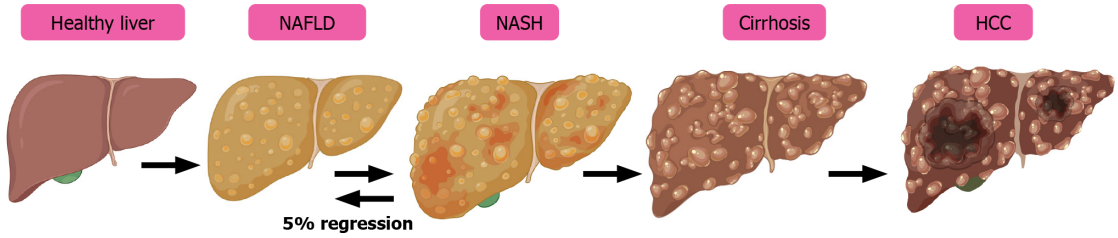
Incretin-based therapies were originally developed for T2D which include GLP-1 receptor agonists (GLP-1 RAs) and dipeptidyl peptidase-4 (DPP-4) inhibitors. GLP-1 RAs (e.g., exenatide, liraglutide, semaglutide) bind to the GLP-1R and increase insulin secretion, inhibit glucagon release, slow gastric emptying, and promote satiety. DPP-4 inhibitors (sitagliptin, saxagliptin, linagliptin) increase the endogenous concentration of GLP-1 by inhibiting the DDP-4 enzyme which degrades GLP-1[44].

NASH is a multi-system disease with underlying metabolic stress driving its manifestations in several organs. The classical progression of NAFLD to NASH to cirrhosis and hepatocellular carcinoma is accelerated in T2D (Figure 3)[45]. Hence agents directed towards underlying metabolic dysfunction like incretin-based therapies could be helpful. These agents have pleiotropic effects apart from regulation of glucose metabolism such as anti-inflammatory and anti-fibrotic effects[46]. Several studies have assessed the clinical efficacy of incretin-based therapies in NASH.

Mechanisms of action of incretin-based therapies in NASH

Insulin sensitivity improvement: GLP-1RAs act through augmenting incretins actions, namely GLP-1. GLP-1 is produced from the gut and has an impact on the pancreas to produce insulin and thus improves IR[47,48].

Effect on central nervous system: GLP-1 also has effects on central nervous system which reduce appetite and can lead to weight loss. GLP-1-based therapies are the mainstay of drug-induced weight loss. Within 6-12 months, semaglutide (a



General population	25%	20%	20% NASH 4% NAFLD	2.8% NASH 0.5% NAFLD
Diabetes mellitus	42%-74%	25%-35%	2 fold higher risk than general population	2-3 fold higher risk

Figure 3 Natural history of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. HCC: Hepatocellular carcinoma; NASH: Nonalcoholic steatohepatitis; NAFLD: Nonalcoholic fatty liver disease.

GLP-1 RA) induces 10%-15% weight loss comparable to bariatric procedures[49].

Anti-inflammatory effects and reduction of liver fat content: GLP-1 receptors are present in pancreas, gastrointestinal tract, CV system, kidney and many others organs. It promotes glucose uptake, and storage of glycogen in the liver and prevents gluconeogenesis[43]. Stimulation of FXR and LXR in the liver using GLP-1 RA may reduce liver fat as shown in animal models/human studies and inflammation.

Anti-Fibrotic Properties: GLP-1RAs like semaglutide have been shown to have dose dose-dependent trend for fibrosis reduction in NASH. With higher doses of GLP-1 RAs, significantly lower fibrosis worsening has been noted highlighting its potential for slowing NASH progression[50].

Improvement in the extra-hepatic milieu in NASH: GLP-1 has many biological effects other than those mentioned above. It has a cardio-protective effect and promotes diuresis/natriuresis in kidneys improving blood pressure (Figure 4). NASH is a multi-system disease with metabolic stress and systemic inflammation[46]. The prerequisites of NASH therapy are that it should improve liver disease and at least be neutral or beneficial with other comorbidities. Incretin-based therapies fulfill these criteria.

Clinical evidence

Several clinical trials have explored the efficacy of incretin-based therapies in NASH (Table 3)[50-64]. The earlier studies were with liraglutide. The landmark LEAN study in 2016 showed that subcutaneous liraglutide 1.8 mg/d led to the resolution of biopsy-proven NASH in 39% of cases compared to 9% in placebo at 48 wk. Moreover, progression of fibrosis was seen in only 9% compared to 36% in placebo. GLP-1 RA also improved body weight, liver enzymes, and liver fat content[55].

GLP-1 RAs also reduce visceral and subcutaneous fat which is not seen with insulin which only affects glycemic control. The reduction in liver fat content with liraglutide was higher than that of pioglitazone[56-60]. Similarly, exenatide 1.8 mg/d reduced liver fat with a higher reduction in body weight, visceral fat, and liver enzymes as compared to insulin glargine[61].

Semaglutide is longer acting GLP-1 RA (half-life 165 h as compared to 13-15 h with liraglutide) which is used as a once-weekly subcutaneous injection (2.4 mg) and is as effective as once-daily liraglutide (3 mg) for weight loss[51,52,65]. However, daily semaglutide with lower doses (up to 0.4 mg) has been used in NASH trials which have shown significant dose-dependent NASH resolution (most pronounced with 0.4 mg) without worsening fibrosis [50,51].

Future directions and implications for NASH management

The tremendous redundancy of the downstream pathways leading to NASH progression warrants combination therapies rather than using single agents. Blocking a single pathway was not shown to be very effective. The best effects are seen with agents that target the underlying metabolic dysregulation with pleiotropic effects such as incretins.

One such approach is combining semaglutide (GLP-1 RA) with small molecules such as firsocostat (acetyl-CoA carboxylase- rate-controlling enzyme for de novo lipogenesis) and cilofexor (second generation farnesoid X receptor agonist). The combination was safe and led to a greater reduction in liver fat compared to semaglutide or a combination of two agents.

GIP reduces ectopic fat accumulation and has anti-inflammatory effects (reduced macrophage polarization and cytokine production). More importantly, GIP ameliorates nausea induced by higher doses of GLP-1 allowing the latter to be used at higher doses. Dual GIP/GLP-1 receptor agonist tirzepatide is being studied in phase III trials for NASH (SYNERGY-NASH). Initial data in T2D and NAFLD showed a significantly higher decrease in liver, subcutaneous, and

Table 3 Summary of trials exploring the efficacy of incretin-based therapies in nonalcoholic steatohepatitis

Ref.	Agent	Comparator	Participants	Duration	Outcomes
Newsome *et al*[50], 2021	Semaglutide 0.1, 0.2 or 0.4 mg s/c once daily	Placebo	NASH with F1, F2, or F3 fibrosis stage	72 wk	NASH resolution without worsening of fibrosis: 40% (0.1 mg), 36% (0.2 mg), 59% (0.4 mg), 33% (placebo); Improvement in fibrosis (P < 0.001 for 0.4 mg *vs* placebo); 43% (0.4 mg), 33% (placebo) (P = 0.48); Weight loss: 13% (0.4 mg), 1% placebo
Loomba *et al*[51], 2023	Semaglutide 2.4 mg s/c	Placebo (1: 2)	NASH with compensated cirrhosis	48 wk	Improvement in liver fibrosis by ≥ 1 stage without worsening NASH: 11% Semaglutide, 29% placebo (P = 0.087); Resolution of NASH at 48 wk: 34% semaglutide, 21% placebo (P = 0.29); No new safety concerns
Romero-Gomez *et al*[52], 2023	Efinopegdutide 10 mg s/c once weekly	Semaglutide 1 mg s/c once weekly	NAFLD with liver fat content (LFC) ≥ 10%	24 wk	Relative reduction in LFC from baseline higher with efinopegdutide (72.7%) than semaglutide (42.3%); Similar reduction in body weight
Alkhouri *et al*[53], 2022	Semaglutide 2.4 mg s/c + cilofexor once daily (30 or 100 mg)+ firsocostat (20 mg)	Semaglutide alone Semaglutide+cilofexor/firsocostat	NASH (MRI-PDFF > 10%, elastography ≥ 7 kPa	24 wk	Combination well tolerated; Greater reduction in liver fat with combination groups *vs* semaglutide (10%-11% *vs* 8.6%); Similar weight loss in all the groups
Flint *et al*[54], 2021	Semaglutide 0.4 mg s/c once daily	Placebo	NAFLD, steatosis ≥ 10% (MRI-PDFF), MRE: 2.5-4.63 kPa	48 wk	≥ 30% reduction in liver steatosis at 24, 48 and 72 wk (64.7%, 76.5%, 73.5%) significantly higher than placebo; Change in liver stiffness in NAFLD not significantly different compared to placebo; Improvement in liver enzymes, body weight and HbA1c
Armstrong *et al*[55], 2016	Liraglutide 1.8 mg/d s/c	Placebo	Biopsy proven NASH	48 wk	39% had resolution of NASH (*vs* 9% placebo); Progression of fibrosis: 9% Liraglutide, 36% placebo
Khoo *et al*[56], 2017	Liraglutide 3.0 mg/d	Diet and moderate exercise	Obesity and NAFLD (Liver fat content > 5% on MRI)	26 wk	Similar reduction in weight, liver fat, liver enzymes in both the groups without any significant difference between the two
Yan *et al*[57], 2019	Liraglutide 1.8 mg/d	Insulin glargine or sitagliptin	T2D and NAFLD	26 wk	Liraglutide and sitagliptin along with metformin reduced body weight, liver fat content, visceral adipose tissue in addition to glycemic control in contrast to Insulin, subcutaneous fat also decreased in liraglutide arm
Bizino *et al*[58], 2020	Liraglutide 1.8 mg/d	Placebo	T2D and NAFLD	26 wk	Liraglutide reduced significantly more body weight and subcutaneous fat but not visceral fat
Guo *et al*[59], 2020	Liraglutide 1.8 mg/d	Insulin glargine or placebo	T2D and NAFLD	26 wk	Liraglutide plus 2 gm metformin for 26 wk significantly reduced liver, subcutaneous and visceral fat
Zhang *et al*[60], 2020	Liraglutide 1.8 mg/d	Pioglitazone	T2D and NAFLD	24 wk	Liraglutide reduced liver fat significantly compare to pioglitazone which could be attributed to weight loss
Liu *et al*[61], 2020	Exenatide 1.8 mg/d	Insulin glargine	T2D and NAFLD	24 wk	Both reduced liver fat but exenatide led to higher reduction in body weight, visceral fat, liver enzymes
Miyake *et al*[62], 2022 (Trial Registration: jRCTs061210009)	Semaglutide 0.5 mg/wk + Luseogli-flozin 2.5 mg/d	Semaglutide 0.5 mg/wk	T2D and NASH	52 wk	Ongoing study
Gastaldelli *et al*[63], 2022	Dual GIP/GLP-1R agonist: tirzepatide10 mg or 15 mg/wk	Insulin degludec	T2D and NAFLD	52 wk	Significant decrease in liver fat content, visceral and subcutaneous adipose tissue compared to insulin degludec
Kuchay *et al*[64], 2020	Glucagon-like peptide-1 receptor	Usual care	T2D and NAFLD	24 wk	Dulaglutide significantly reduces liver fat comma gamma glutamyl

(GLP-1r) agonist (GLP-1 RA)	transpeptidase

GIP: Glucose-dependent insulinotropic polypeptide; MRE: Magnetic resonance elastography; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; PDFF: Proton density fat fraction.

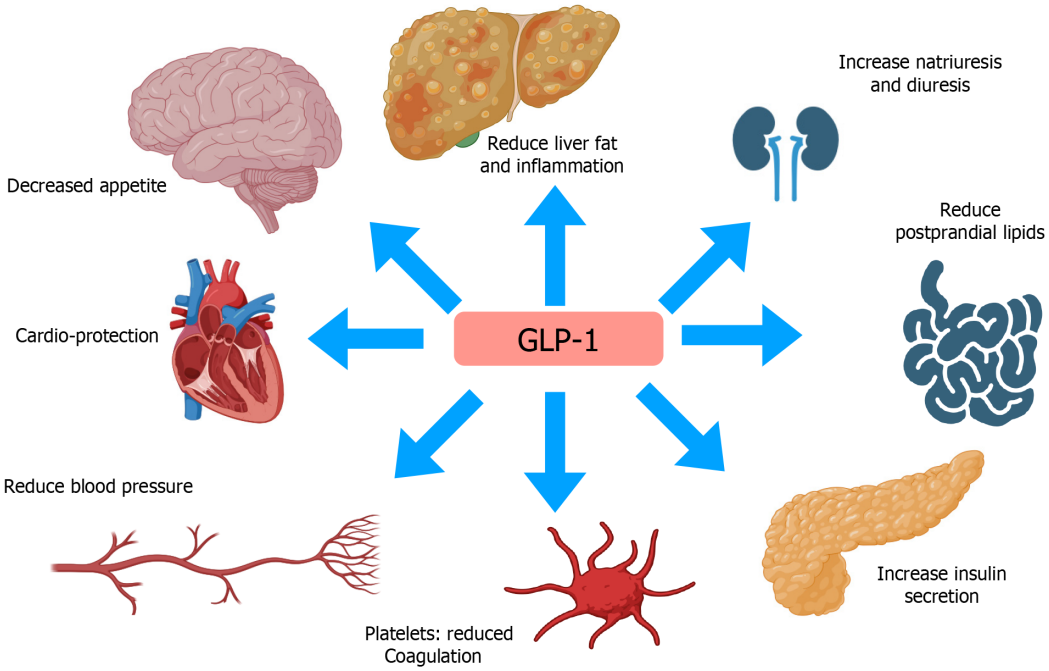


Figure 4 Pleotropic effects of glucagon-like peptide 1 and its effect of several organ systems in systematic inflammation characteristic of nonalcoholic steatohepatitis. GLP-1: Glucagon-like peptide 1.

visceral fat as compared to insulin degludec[63]. Once weekly semaglutide (0.5 mg/wk) is being studied with sodium glucose co-transporter 2 inhibitors (Luseogliflozin 2.5 mg/d) for T2D with NASH[62].

GLP-1 is also being combined with glucagon for NASH. Glucagon has direct liver receptors whereas effects of GLP-1 RAs are based on extra-hepatic effects. Glucagon has hepatic receptors where it has anti-inflammatory effects, reduces bile acid production/hepatic stellate cell activation, inhibits transforming growth factor signaling, reduces collagen production, and increases turnover. Dulaglutide, a GLP-1 RA, is being studied in NASH. Earlier studies in T2D and NAFLD showed a reduction in liver fat[64]. GLP-1/glucagon receptor co-agonist efinopegdutide 10 mg weekly resulted in a greater reduction in liver fat than weekly semaglutide (1 mg) in a phase IIa study[52]. Histology-based trials in NASH are warranted.

Challenges and promise of incretin-based therapies for NASH

Selecting the right category of patients for incretin-based therapy needs further exploration. Disease activity (inflammation), stage (fibrosis), and comorbidities (*e.g.*, T2D) need to be considered. The long-term safety of these therapies in NASH needs further study. The holistic approach of combination treatment including incretin-based therapies beyond glycemic control is a potential paradigm shift in NASH management and is an area of ongoing research. Collaborative efforts among healthcare providers such as primary care doctors, hepatologists, and endocrinologists are warranted.

CONCLUSION

In conclusion, specific targeted interventions are essential for NAFLD in T2D. This is because of the alarmingly high prevalence of NAFLD in T2D, their synergistic effect on hepatic and extra-hepatic complications, and the bidirectional link driven by alterations in glucose/Lipid metabolism along with insulin resistance. PPAR agonists such as pioglitazone have been shown to improve liver outcomes by regulating lipid metabolism, glucose homeostasis, and inflammation. Newer dual and pan-PPAR agonists show promising results with minimized side effects in NAFLD/NASH with T2D. Incretin-based therapies, (GLP-1RAs and DPP-4 inhibitors) act in T2D-related NAFLD/NASH by glycemic control as well their exhibit pleiotropic effects such as anti-inflammatory and anti-fibrotic actions. Liraglutide and semaglutide have demonstrated remarkable efficacy in resolving NASH without worsening fibrosis apart from improving metabolic parameters. Given the multifactorial nature of NASH pathogenesis, a combination of incretin-based therapies with small

molecules targeting de novo lipogenesis and sodium glucose co-transporter 2 inhibitors are upcoming approaches for treating NASH. Dual GLP-1/glucagon receptor agonists with direct hepatic effects of glucagon affecting fibrosis could indicate a paradigm shift in NASH management. The current challenges include the proper selection of patients for incretin-based therapies, concerns regarding long-term safety, and the need for a multifaceted approach. As we better understand the intricate interplay between T2D and NAFLD, PPAR agonists and incretin-based therapies offer hope for more effective and targeted management of NAFLD/NASH in T2D.

FOOTNOTES

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Country/Territory of origin: India

ORCID number: Subhodip Pramanik 0000-0002-3196-1192; Partha Pal 0000-0002-7090-9004; Sayantan Ray 0000-0002-6274-465X.

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MINIREVIEWS

Novel automated non-invasive detection of ocular surface squamous neoplasia using artificial intelligence

Sony Sinha, Prasanna Venkatesh Ramesh, Prateek Nishant, Arvind Kumar Morya, Ripunjay Prasad

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Sony Sinha, Department of Ophthalmology–Vitreo Retina, Neuro Ophthalmology and Oculoplasty, All India Institute of Medical Sciences, Patna 801507, India

Prasanna Venkatesh Ramesh, Department of Glaucoma and Research, Mahathma Eye Hospital Private Limited, Trichy 620017, India

Prateek Nishant, Department of Ophthalmology, ESIC Medical College, Patna 801113, India

Arvind Kumar Morya, Department of Ophthalmology, All India Institute of Medical Sciences, Hyderabad 508126, India

Ripunjay Prasad, Department of Ophthalmology, RP Eye Institute, Delhi 110001, India

Corresponding author: Arvind Kumar Morya, MBBS, MNAMS, Additional Professor, Department of Ophthalmology, All India Institute of Medical Sciences, Bibi Nagar, Hyderabad 508126, India. bulbul.morya@gmail.com

Abstract

Ocular surface squamous neoplasia (OSSN) is a common eye surface tumour, characterized by the growth of abnormal cells on the ocular surface. OSSN includes invasive squamous cell carcinoma (SCC), in which tumour cells penetrate the basement membrane and infiltrate the stroma, as well as non-invasive conjunctival intraepithelial neoplasia, dysplasia, and SCC in-situ thereby presenting a challenge in early detection and diagnosis. Early identification and precise demarcation of the OSSN border leads to straightforward and curative treatments, such as topical medicines, whereas advanced invasive lesions may need orbital exenteration, which carries a risk of death. Artificial intelligence (AI) has emerged as a promising tool in the field of eye care and holds potential for its application in OSSN management. AI algorithms trained on large datasets can analyze ocular surface images to identify suspicious lesions associated with OSSN, aiding ophthalmologists in early detection and diagnosis. AI can also track and monitor lesion progression over time, providing objective measurements to guide treatment decisions. Furthermore, AI can assist in treatment planning by offering personalized recommendations based on patient data and predicting the treatment response. This manuscript highlights the role of AI in OSSN, specifically focusing on its contributions in early detection and diagnosis, assessment of lesion progression, treatment planning, telemedicine and remote monitoring, and research and data analysis.

Key Words: Conjunctival neoplasm; Early detection of cancer; Machine learning; Deep neural network; Precision medicine

Core Tip: This is a unique comprehensive review written on a common eye tumour-ocular surface squamous neoplasia (OSSN) and the role of artificial intelligence (AI) in the early diagnosis and management of this ocular condition. This write-up also covers the various intricacies involved in developing AI algorithms based on digital and histopathological images of OSSN.

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INTRODUCTION

Artificial intelligence (AI) is the development of human or better than human abilities incorporated in computers, digital devices and machines to reduce quantum of error in the overall performance in a particular dimension. Since 1940, there has been rapid advancement in the utilization of AI in medical sciences, more so in the formulation of algorithms for effective diagnosis and management of various diseases. Ocular surface squamous neoplasia (OSSN) is among the common eye tumours responsible for considerable ocular morbidity causing sight-threatening complications if not diagnosed early and managed accordingly. Advanced invasive lesions may need orbital exenteration, which carries a risk of death. This article provides a comprehensive review of the deep and intricate insights regarding the role of AI in the non-invasive detection of OSSN, and presents the practical possibilities related to the future of AI in OSSN.

SEARCH METHODOLOGY

This review article was written after a literature search on EMBASE, Google Scholar, PubMed, Scopus, Web of Science and Cochrane library database. Only highly cited articles on OSSN and AI published between 1932 and 2022 in the English language were included. *Reference Citation Analysis* was also used to assess the articles on AI, relying upon the impact index per article to emphasize the latest published articles. The articles were reviewed by five independent researchers and the relevant literature compiled.

OSSN

OSSN is an umbrella term used to include all primary epithelial dysplastic and carcinomatous lesions of the ocular surface (Table 1). It includes conjunctival or corneal intraepithelial neoplasia [conjunctival intraepithelial neoplasia (CIN): Wherein the neoplastic cells are confined to the epithelium and do not breach the basement membrane], as well as invasive squamous cell carcinoma (SCC) (invasive SCC: When the basement membrane is breached and neoplastic cells invade across it)[1-5].

The overall incidence of OSSN has considerable geographical variation[6]. The incidence of invasive SCC varies from 0.02/100000 to 3.5/100000 population, and 75% of those affected are elderly, 75% are men and the lesion is limbal in 75% of the cases[7]. Low-income countries demonstrate the young, female demographic in OSSN prevalence, the higher-income demonstrate the older male demographic, with middle-income countries falling between the two with an intermediate age and no sex predilection[8]. In a recent study in India, the mean age at diagnosis was about 50 years[9]. In the elderly, they are the third most common type of oculo-orbital tumours, being exceeded in frequency only by melanoma and lymphoma[1]. OSSN poses a considerable economic burden, given the potentially high out-of-pocket costs for topical chemotherapy, lost work time for follow-up visits, the cost of the office visits, and even transportation costs to the visits[10].

DIAGNOSIS OF OSSN

Initial method of diagnosis

The diagnosis of OSSN was initially entirely clinical. Patients with OSSN may be asymptomatic (30%) or present with non-specific symptoms like grittiness, redness, irritation and/or a mass in the eye which correlate poorly with the extent

Table 1 Entities included in the term 'ocular surface squamous neoplasia'

Term	Description	Structures included	First introduced
Epithelioma[1]	A generalized term encompassing neoplastic proliferation of the ocular surface epithelium, subsequently identified as squamous cell carcinoma of the conjunctiva and cornea	Conjunctiva and cornea	1860
Conjunctival Intraepithelial Neoplasia[2]	Abnormal neoplastic tissue involving the epithelium of the conjunctiva alone or the cornea as well	Conjunctiva and cornea	1978
Corneal Intraepithelial Neoplasia[2,3]	Disordered epithelial maturation (dysplasia) associated with abnormal growth of the corneal epithelium	Cornea	1984
Conjunctival and corneal invasive neoplasia[4]	Invasion of abnormal neoplastic tissue involving the epithelium of the conjunctiva or cornea	Conjunctiva or cornea	1986

or depth of the lesion[5,11]. Multiple non-specific terms were used to describe the various presentations on observation of the ocular surface under direct illumination and magnification using a loupe or slit-lamp biomicroscope. The possibility of OSSN is suggested by a slightly elevated, usually well-demarcated fleshy lesion at the limbus with conjunctival part having a gelatinous, leukoplakic, or papillary appearance and feeding blood vessels usually in the interpalpebral area, a description which overlaps with several common inflammatory, dystrophic, or cicatricial disorders of the epibulbar region leading to wide differential diagnoses[12-14]. Similarly, corneal OSSN appears as an area of translucent clouding, which may not suggest a diagnosis of neoplasia at all[5]. In addition, due to varied differential diagnoses, it was not possible to rely entirely on history and clinical observation alone to exclude the possibility of malignancy in various ocular surface lesions, leading to the evolution of histopathologic methods for OSSN diagnosis[15].

Invasive methods of diagnosis

Biopsy with classical staining methods was first done in 1932 where features of an OSSN lesion were studied and described to be of a papilloma[16]. However, McGavic and Stout studied the sections again, and identified it as corresponding to CIN. They concluded that this was the first report of histopathologically-proven Bowenoid epithelium occurring in the eye[17]. Ever since, histopathology remains the gold standard for confirmatory diagnosis of OSSN, with the use of staining of the specimen with vital dyes such as rose bengal, toluidine blue (0.05% or 1%) or methylene blue (1%) being a recent additional improvement[15,18-22]. The histopathological features were found to help in prognostication of OSSN and identify invasion of the basement membrane.

With the identification of viral associations of OSSN, efforts were directed to identify various viral etiological factors in OSSN which may coexist especially in immunodeficient patients[23]. Thus, Southern blot and in-situ hybridization (ISH) were then introduced as methods to detect human papillomavirus (HPV) infection in histopathological sections of ocular surface tumours in the 1980s[24,25]. It is of considerable interest, that it was found that ISH can distinguish reactive atypia and true dysplasia in conjunctival tumours[26]. However, RNA in formalin-fixed tissue is labile and likely to affect the sensitivity of ISH[27]. Only a weak association was found between HPV infection and OSSN[28].

Immunostaining for markers for proliferative potency and cell differentiation such as p16 and nuclear antigen Ki-67, mutant tumor suppressor gene p53, and argyrophilic nucleolar organizer regions was shown to be useful in detecting and prognosticating malignancy of conjunctival lesions including OSSN[29]. Preferential oncogene p63 expression has been demonstrated in anaplastic conjunctival epithelial cells with this technique[30]. However, these immunohistochemical approaches are prone to false-positive results as suggested recently[26].

Optical biopsy using a novel autofluorescence multispectral imaging technique has been recently described which produces false colour images from histopathological sections of ocular surface samples to enable identification of OSSN based on spectral signatures[31,32]. This is a promising technique for the identification of OSSN, which has a potential for automation. However, this too, cannot overcome the limitations of the excisional biopsy itself.

Excision biopsy is limited by the inability to determine orientation of the samples and subjectivity of the interpretation of histopathological findings. Lesions not included in the excised tissue may be missed as poorly demarcated diffuse lesions can be difficult to excise completely[27]. Moreover, with recurrent OSSN, repeated excisional biopsies may lead to conjunctival scarring and limbal stem cell deficiency[33]. The presence of negative histopathological margins is no guarantee for preventing the recurrence of OSSN[34]. This led to the exploration of non-invasive methods in the diagnosis of OSSN.

Non-invasive methods of diagnosis

Direct dye staining of the lesion with vital dyes enables identification of OSSN *in vivo*, in very small lesions and in those coexisting with atopy and pterygium. However, dyes like lissamine green and rose bengal used to delineate the extent of OSSN lesions are nonspecific and stain many other ocular surface conditions[27]. Toluidine blue and methylene blue staining have high sensitivity but low to moderate specificity in diagnosing OSSN[20-22]. Thus, they are good as screening tools but are an insufficient diagnostic modality.

Exfoliative cytology of conjunctival surface tumours had been popularized using platinum spatula, brush, and cotton-wool tipped swabs since 1954[35,36]. Shortcomings of exfoliative cytology included patient discomfort, inability to localize the pathology or study the degree of tumor invasion and altered cell-to-cell relationships causing inability to

pinpoint the diagnosis of OSSN and not enabling differentiation between invasive and non-invasive disease[2]. Similar problems were encountered with aspiration cytology, in which cells were drawn from the conjunctival surface using a fluid-filled syringe[15]. It was in 1994 that impression cytology was employed to describe the diagnostic cytological findings and cell-to-cell relationships in cases of conjunctival tumours. Detailed cytomorphology of OSSN was then described in 2001[37]. Superficial epithelial cells can be collected by cellulose acetate paper, biopore membrane or millipore cellulose acetate filter paper to which the cells adhere and are lifted off the ocular surface. Impression cytology satisfactorily preserves the cytologic features, while overcoming the limitations of overlapping cells in exfoliative cytology specimens[38-40]. Cytology has an overall sensitivity of 72.4% and a specificity of 74.3%, and a considerable proportion of cytology specimens may have to be excluded from analysis due to inadequate cellularity[41]. Impression cytology has been found to be positive in only 77% to 80% of cases as it can assess only superficial cells and is unable to sample deep lesions or invasive disease[40]. It is not possible to differentiate intraepithelial lesions from invasive SCC given the superficial sampling of cells in this technique[37,42,43]. Moreover, it requires considerably skilled professionals to interpret results. A fair negative predictive accuracy indicates that impression cytology is a valuable screening technique, but not a gold standard[42]. Staining of cytology specimens with vital dyes is another improvement, however, it could not overcome the low specificity that makes it only a good screening tool[18-22].

In 2002, Char *et al*[44] first reported ultrasound biomicroscopy (UBM) for studying SCC of the ocular surface with intraocular extension. On UBM, the surface of the OSSN is hyperechoic while the interior is hypoechoic. It was found to be the most useful tool to assess and document intraocular extension of OSSN, wherein blunting of the anterior chamber angle and uveal thickening are evident findings which correlate well with histopathological observations[45]. UBM requires a water bath in the reclined position and a technician familiar with its use to obtain the best images. Moreover, for studying intraocular invasion of OSSN, it requires two probes: A 50 MHz probe for images that have better resolution, and a 20 MHz UBM to provide deeper and wider field of view. However, definition of the posterior margins of the tumor is limited by maximum penetration of both 20 MHz and 50 MHz UBM[45]. Images have lower resolution as compared to the newer modalities[27].

Duchateau *et al*[46] first applied *in vivo* confocal microscopy (IVCM) to assess CIN in 2005. Subsequently, various authors described different features of OSSN on IVCM, some of which could provide microscopic evidence of corneal involvement[47]. IVCM can identify cellular anisocytosis, anisonucleosis, altered nuclear:cytoplasmic ratio, and nests of isolated keratinized mitotic cells extending beyond the basement membrane (invasion)[48]. Several distinctive features such as large nucleoli with starry night appearance and the absence of sub-basal corneal nerves make IVCM diagnostic for OSSN, having complete agreement with scraping cytology and histology[49,50]. However, the instrument is limited in availability, and the quality of imaging relies on the illumination of the object and reflected light. Ocular surface tumours have poor optical properties given the keratinization, especially the leukoplakic types. And several features of benign and malignant lesions on IVCM overlap, making it difficult to guide treatment decisions[15]. Confocal microscopy requires a skilled technician to perform the test[27]. Moreover, it is neither possible to determine the presence of microinvasion by this method, nor is it possible to reliably differentiate benign from OSSN lesions due to overlap of IVCM features in various ocular pathologies[46]. Additionally, it is difficult to obtain IVCM images and biopsy specimens from the exact same site where the tissue is being examined[51]. Another limitation is that it can provide only en-face images in contrast to cross-sectional images obtained in tissue histology[50]. Moreover, because it provides images at a cellular level, IVCM is unable to provide a comprehensive scan of the entire ocular surface[27].

Anterior segment optical coherence tomography shows epithelial thickening and increased reflectivity of epithelium with an obvious delineation of OSSN-affected tissue, which correlates well with the gold standard histopathologic examination in differentiating OSSN from other corneal and conjunctival pathologies to guide appropriate management and monitor treatment response[15,52,53]. Microscopic detail to the extent of a 2 μm resolution was achieved with an ultrahigh-resolution optical coherence tomography (UHR-OCT) device in 2011 and its role in monitoring the disease, detection of recurrence and guiding the withdrawal of treatment has been well documented[33]. UHR-OCT is useful when clinical differential diagnosis of ocular surface lesions are broad, not only in the case of small limbal OSSN which are frequently misdiagnosed as pterygia, but also in the case of corneal OSSN which can be clinically confused with Salzmann nodular degeneration, vascularized corneal scars, melanomas, sequelae of limbal stem cell deficiency, herpes simplex viral keratopathy, atypical peripheral corneal infiltrates (suspicious for vernal keratoconjunctivitis) and other complex ocular surface pathology which may even coexist with OSSN[54]. Optical signs indicating complex, subtle, atypical or residual OSSN are revealed by UHR-OCT which shows tremendous promise for the non-invasive diagnosis of OSSN[33,54]. The UHR-OCT is, however, a custom built machine, and is not commonly available[27].

The use of commercially available, HR, spectral-domain OCT in 2015 was a giant leap for the non-invasive diagnosis of OSSN. OCT can be used to measure epithelial thickness, which differentiates OSSN from pterygium with an 87%-100% sensitivity and 75%-100% specificity[41,52]. OCT angiography imaging on newer OCT devices allows for visualization and quantification of vessel structure and density within, under, and surrounding OSSN[55]. Mean vessel area density in the subepithelium adjacent to the OSSN increases with treatment, then decreases significantly between mid-treatment and resolution[56]. This can be useful in evaluating the effect of medical therapy on OSSN resolution.

Modern OCT modalities are not without limitations as they are less useful in evaluating pigmented lesions[52]. There are no consistent criteria for distinguishing CIS from invasive SCC on OCT. The presence of feeder vessels, intrinsic vascularity and a nodular lesion raise suspicion of invasive SCC, but there are no objective or quantitative criteria regarding their presence on OCT which may suggest invasion. In addition, the depth of invasion of OSSN cannot be reliably assessed due to poor penetration of the OCT[57]. OCT angiographic studies are limited by artifacts created by patient movements. Thick tumors and leukoplakia preclude detailed visualization of blood vessels especially in the subepithelial tissue. The relationships between baseline and change in vessel characteristics and tumor grade and stage are poorly characterized. In addition, there are difficulties in image processing, including manual segmentation of the

tumors, projection artifacts, and vascular density calculations[56].

The multiple modalities and imaging data from invasive and non-invasive methods of detection of OSSN are complex to interpret and correlate with patient characteristics, history and clinical examination findings, response to treatment, follow-up and final outcomes. These results can be observed by humans as well as AI algorithms, which raises the possibility of automating the diagnosis of OSSN.

WHAT IS AI

AI has emerged as a powerful tool in various fields, and its potential in OSSN diagnosis is particularly promising. With its ability to process vast amounts of data and identify complex patterns, AI has the potential to revolutionize non-invasive objective diagnosis of OSSN based on automation, and improve patient outcomes. This is possible using various component systems working together to function like human intelligence, while avoiding the factors of subjectivity, fatigue and bias.

COMPONENTS OF AI

Neural networks

Neural networks enable computers to perform complex tasks once thought to be the exclusive domain of human intelligence. Two prominent architectures of artificial neural networks (ANN) and convolutional neural networks (CNN) have made significant contributions to the field of machine learning, enabling breakthroughs in the diagnosis of breast cancer, skin cancer, prostate cancer, lung cancer, *etc*[58].

ANN are inspired by the structure and function of biological neural networks in the human brain. ANN consist of interconnected nodes, called artificial neurons or perceptrons, organized into layers, the three main types of layers being the input layer, hidden layers, and output layer. Each neuron receives inputs, applies an activation function, and passes the result to the perceptrons in the next layer. Through a process called backpropagation, neural networks can learn from data and adjust the weights of their connections to improve their performance.

CNN, on the other hand, are specifically designed for processing grid-like data such as images, and excel in tasks that require extracting meaningful features from input data. They use a specialized type of convolutional neuron to perform convolution operations on the input data. CNN typically consist of multiple layers, including convolutional layers, pooling layers, and fully connected layers. The convolutional layers extract local patterns or features from the input data, while the pooling layers downsample the feature maps to reduce the spatial dimensions. The fully connected layers at the end of the network perform classification or regression tasks based on the extracted features.

The strength of ANN lies in its ability to solve a wide range of problems, including regression, classification, and sequence generation enabling tasks like image recognition. On the other hand, CNN have demonstrated exceptional performance in computer vision tasks, such as object detection, image segmentation, and image classification due to the ability to capture local patterns and spatial hierarchies. These have already been successful in classifying cancerous lesions in organs other than the eyes, thus building promise for their use in OSSN[59].

Deep learning

Deep learning focuses on training ANN with multiple layers, known as deep neural networks, which mimic the complex interconnections and hierarchical representations found in the human brain. Deep learning algorithms learn directly from raw data by automatically extracting features and patterns through multiple layers of computation. These layers allow for increasingly abstract representations of the input data, enabling the network to learn hierarchical features and make more accurate predictions or decisions. One of the key advantages is its ability to handle large-scale, high-dimensional data. As of today, various types of medical images, such as those of radiological investigations have been used to develop deep learning models for cancers of the lung, rectum, pancreas, stomach, prostate, brain, breast, *etc*[60].

Data augmentation

Data augmentation is a technique widely used in machine learning and deep learning to increase the size and diversity of a training dataset by creating new samples through various transformations. It involves applying random modifications to the existing data, such as rotations, translations, flips, zooms, or changes in brightness and contrast. Its purpose is to improve the generalization and robustness of machine learning models. It is particularly useful in scenarios where the available labelled data is limited or imbalanced. By providing additional variations of the training data, it helps the model learn more invariant and discriminative features, reducing overfitting and enhancing the model's ability to handle unseen data.

Commonly used in computer vision tasks, such as image classification or object detection, data augmentation can also be applied to other types of data. By artificially increasing the size and diversity of the training dataset, this serves as a regularization technique enabling improved performance and better utilization of available data resources.

Pre-trained networks

Pre-trained networks are ANN that have been trained on large-scale datasets for specific tasks, such as image classification or natural language processing. The concept behind these networks is to leverage the knowledge and features

learned from one task and transfer them to another related task. By initializing the network with pre-trained weights, the model already possesses some understanding of the underlying patterns in the data, saving significant training time and resources. Hence, they can achieve state-of-the-art performance on various tasks, even with limited labelled data. They also facilitate faster experimentation and prototyping, as researchers can focus on fine-tuning the model for specific tasks rather than starting from scratch.

Common pre-trained network models like very deep neural networks, deep residual networks, and bidirectional encoder representation from transformers have been pre-trained on large-scale datasets like ImageNet, capturing general features and patterns. Researchers and practitioners can then adapt these pre-trained models by adding or modifying layers to suit their specific task or domain.

Feature extractors

Feature extractors are algorithms or components within machine learning systems responsible for capturing and representing meaningful patterns or features from raw data. This is important when dealing with high-dimensional or complex data, such as images, audio, or text. They can reduce the dimensionality of the data, remove noise or irrelevant information, and highlight discriminative characteristics essential for the learning process. Feature extractors can be handcrafted, or they can be learned automatically through deep learning techniques.

Transfer learning

Transfer learning is a machine learning technique that leverages knowledge and models learned from one task or domain to improve performance on a different but related task or domain. Pre-trained models trained on large datasets for a specific task can be adapted to new tasks or datasets. Transfer learning can significantly reduce the amount of labelled data required for training, making it useful when labelled data is scarce or expensive to obtain. Overall, it enables researchers and practitioners to transfer knowledge and representations across tasks, leading to improved performance, faster training, and better utilization of available resources. As of today, transfer learning is being widely used in interpretation of retinal scans obtained by modern imaging techniques like optical coherence tomography angiography [61].

Algorithms of qualitative and quantitative analysis

AI algorithms are increasingly being utilized for both qualitative and quantitative analysis in various domains. In qualitative analysis, AI algorithms can be used to analyze unstructured data, such as text or images, and extract relevant information. Computer vision algorithms can interpret images and videos, enabling object recognition, and image classification. On the other hand, AI algorithms play a crucial role in quantitative analysis by processing structured data and performing statistical analyses. These can identify patterns, correlations, and trends as well as perform predictive modelling, regression analysis, and clustering, providing valuable insights into complex datasets. AI algorithms also enable the integration of qualitative and quantitative analyses by combining different types of data.

ROLE OF AI IN OSSN

One of the key challenges in OSSN diagnosis is the accurate interpretation of medical images, such as anterior segment photographs, OCT images, computed tomography and magnetic resonance imaging scans, and those obtained by other *in vivo* techniques. AI algorithms can analyze these non-invasively acquired images with incredible speed and precision, assisting radiologists, pathologists and ophthalmic surgeons in detecting subtle abnormalities that may indicate the presence and extent of neoplasia. AI can also help in distinguishing benign lesions from malignant tumours, reducing the need for unnecessary biopsies and surgeries.

AI can aid in early detection of OSSN, leading to better treatment outcomes. By analyzing patient data, including genetics, medical history and lifestyle factors, the AI algorithms can identify individuals at a higher risk of developing OSSN and determine the interacting risk factors in a patient with a suspicious lesion. This enables proactive screening and early intervention, when the disease is more treatable and potentially curable.

Another area of great promise is in the development of personalized treatment plans. OSSN is a complex disease with significant inter-patient variability. AI algorithms can integrate patient-specific data and clinical knowledge to predict treatment responses and recommend tailored therapies. This can optimize treatment decisions between various possible approaches, improve rates of treatment success, and minimize unnecessary side effects.

Furthermore, AI-powered tools can enhance the efficiency and accuracy of molecular profiling crucial for precision medicine. AI algorithms can analyze large genomic datasets, identify genetic mutations or biomarkers associated with specific cancers, and suggest targeted therapies or clinical trials. This will enable ocular oncologists to make more informed decisions about the most effective treatment options for individual patients.

AI can also assist in monitoring treatment responses. By continuously analysing images, laboratory results, and real-time physiological data, AI algorithms can detect early signs of treatment failure or disease progression. This facilitates timely adjustments to treatment plans, ensuring optimal outcomes.

Automated screening

Automated screening aims to detect and identify suspicious lesions on the ocular surface[62]. The integration of AI in automated screening for OSSN brings several advantages (Table 2).

Table 2 Various advantages of integrated artificial intelligence in automated screening

Advantages of automated screening by AI for OSSN
It enables the screening process to be more efficient and objective, reducing the reliance on subjective human interpretation
AI algorithms can analyze large volumes of images rapidly and consistently, aiding in the early identification of suspicious lesions that may otherwise be overlooked
Automated screening can enhance access to care, particularly in areas where specialized ophthalmic expertise may be limited, through telemedicine and remote monitoring
By providing a preliminary assessment of OSSN lesions, AI technology can support primary care providers and community healthcare workers in triaging patients and referring those in need of further evaluation to specialized centers
AI-driven automated screening holds promise in improving the early detection and management of OSSN, ultimately leading to better patient outcomes

AI: Artificial intelligence; OSSN: Ocular surface squamous neoplasia.

AI can highlight areas of potential abnormality in the ocular surface for further evaluation. By leveraging machine learning techniques, AI models can learn from a vast dataset of annotated images to accurately differentiate OSSN lesions from normal ocular tissue[63].

Prediction

AI models can analyze clinical and molecular data to predict outcomes such as treatment response, disease progression, and recurrence. By considering patient-specific factors and integrating multimodal data, AI can generate personalized predictions that enable clinicians to optimize treatment strategies, adjust follow-up schedules, and tailor interventions based on the individual characteristics of each patient, ultimately improving the overall management and prognosis of OSSN[64].

By analyzing genetic data from tissue samples, AI algorithms can identify patterns and correlations that may be indicative of aggressive disease or potential treatment targets[65]. This molecular profiling combined with predictive modelling could enable a deeper understanding of the underlying mechanisms of OSSN and facilitate the development of targeted therapies.

Furthermore, AI predictions may assist in stratifying OSSN subtypes, which may have different clinical behaviours and responses to treatment. This stratification can allow for more precise and personalized therapeutic approaches, potentially reducing unnecessary interventions and improving patient outcomes[51].

Deep learning models for automated diagnosis

Deep learning models based on ANN with multiple layers, can analyze large amounts of data, including images, clinical features, and molecular information, to make accurate and efficient diagnostic predictions for OSSN. By training on annotated datasets of OSSN cases, deep learning models can learn complex patterns and features that are indicative of the disease, enabling them to differentiate between normal ocular tissue and OSSN lesions (Figure 1). This holds great potential for improving diagnostic accuracy, reducing interobserver variability, and assisting healthcare professionals in making informed decisions[66].

In the case of OSSN, deep learning models can analyze images of the ocular surface to detect irregularities in tissue morphology, abnormal vascular patterns, and other features that may be challenging for human observers to discern. By leveraging CNN which are particularly adept at image analysis, deep learning models can automatically extract relevant features from OSSN images and provide objective and consistent diagnostic assessments[48].

Automated classification and staging

AI algorithms can be trained to accurately classify OSSN lesions into different subtypes based on their characteristics, such as size, location, and extent of involvement. By analyzing clinical and imaging data, including photographs, OCT scans, and histopathological features, AI models can categorize OSSN lesions into distinct subtypes, such as intraepithelial neoplasia, invasive SCC, or carcinoma in situ[67].

Furthermore, AI-based automated staging of OSSN lesions can aid in determining the extent and severity of the disease. By analyzing imaging data and clinical parameters, AI models can predict the stage of OSSN, which refers to the depth of invasion and potential spread of the neoplastic cells. Thus, it helps identify cases that may require more aggressive interventions, such as surgical excision or adjuvant therapy. By accurately predicting the stage of OSSN, AI models can assist clinicians in developing personalized treatment plans[33].

Evaluation of severity

AI-based evaluation of severity in OSSN offers several benefits (Table 3). AI algorithms can analyze various clinical and imaging data to assess the severity of OSSN lesions accurately. By utilizing machine learning techniques, AI models can learn from annotated datasets and develop algorithms that consider factors such as lesion size, location, extent of invasion, and involvement of adjacent structures. This comprehensive evaluation of severity aids in determining the appropriate treatment approach, including the need for surgical intervention, adjuvant therapy, or conservative management[68].

Table 3 Various advantages of artificial intelligence-based evaluation of severity in ocular surface squamous neoplasia

Advantages of AI-based evaluation of severity in OSSN
It provides an objective and standardized assessment, reducing interobserver variability that may be present in traditional evaluation methods AI algorithms can analyze large amounts of data rapidly and consistently, ensuring accurate and reproducible severity evaluation
Additionally, AI can incorporate multi-modal data, including imaging findings from techniques such as OCT, confocal microscopy, or histopathological characteristics
This integration of diverse data sources enhances the accuracy and reliability of severity evaluation, enabling clinicians to make informed decisions regarding treatment planning and prognostication
Overall, AI-driven evaluation of severity in OSSN holds promise in improving patient outcomes by facilitating appropriate and tailored management strategies based on the individual characteristics of each case

AI: Artificial intelligence; OCT: Optical coherence tomography; OSSN: Ocular surface squamous neoplasia.

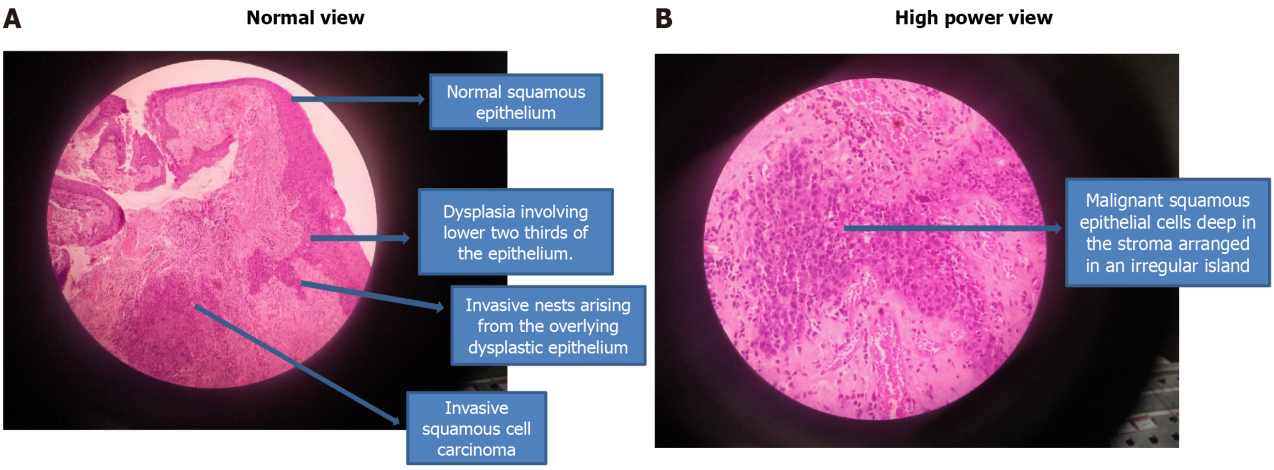

Figure 1 Typical features of ocular surface squamous neoplasia that can be recognized by artificial intelligence. A: Microphotograph showing normal squamous epithelium in the upper part along with dysplasia involving lower two-thirds of the epithelium and Invasive squamous cell carcinoma; B: Microphotograph in high magnification showing malignant squamous epithelial cells deep in the stroma arranged in an irregular island.

Patient experience and satisfaction

Patients who undergo diagnostic evaluation using non-invasive AI-based tools in conjunction with expert clinical decision-making by ocular oncologists are likely to experience more rapid, reliable, and robust interpretation of their clinical condition, assurance of a better clinical response to treatment modalities selected and a more complete explanation of the prognosis and risk of recurrence, leading to better patient experience. However, enforcing AI-based treatment decisions may undermine the participation of the patient in the decision-making process, leading to poor acceptance of these treatment modalities and follow-up regimens, with overall less satisfaction and potential drop-out or loss to follow-up. For example, an AI algorithm might predict that a certain OSSN may respond better to Mitomycin C drops compared to interferon alpha 2B drops, but the ocular surface irritation caused by mitomycin drops may lead to poor compliance to this therapy leading to poor outcome and less patient satisfaction[62].

LIMITATIONS AND CHALLENGES

Despite the numerous advantages of AI in OSSN, certain limitations and challenges need to be acknowledged. Firstly, the availability and quality of data are crucial for training AI models. In the case of OSSN, obtaining large, diverse, and well-annotated datasets can be challenging, as the condition is relatively rare. Limited data availability may affect the performance and generalizability of AI algorithms. Additionally, the development and validation of robust AI models requires substantial computational resources, technical expertise, and rigorous testing. Ensuring the reliability, accuracy, and safety of AI algorithms is of utmost importance to avoid potential errors or biases. Multicentric image acquisition with centralized image processing and expert interpretation may be useful to train the data sets to develop the AI models. Identification of key centres and international collaborations to include diverse ethnic groups should be the first step towards overcoming these challenges.

Ethical considerations, such as privacy concerns and the responsible use of patient data, must be addressed when implementing AI in OSSN. The concerns encompass breach of patient identity, hacking of ocular signatures such as iris patterns, and even misuse of genetic information. To prevent attacks such as these, data must be processed through the

use of secure and encrypted channels, and the results transferred in an anonymized manner to protect patient identity and privacy. Research should explore how cloud computing and blockchain technology can be made useful for addressing concerns of data security in this regard[69].

Furthermore, the integration of AI technology into clinical practice requires proper validation, training, education, and acceptance from healthcare professionals to effectively leverage its benefits through collaborative efforts among clinicians, researchers, and AI experts. Research and publications of increasingly higher levels of evidence will help to overcome the concerns about shortcomings of AI models and improve their reliability and acceptance.

EVOLUTION OF AI DIAGNOSIS

Computer assisted image analysis

Computer-assisted image analysis is the first step towards application of AI in OSSN. By leveraging AI algorithms, computer systems can analyze images of the ocular surface to aid in the detection, characterization, and monitoring of OSSN lesions in large populations[70,71]. This technology enables enhanced visualization and analysis of OSSN lesions, facilitating early detection, precise diagnosis, and improved treatment planning[34].

One of the key advantages of computer-assisted image analysis is its ability to detect subtle and early changes in OSSN lesions that may not be apparent to the human eye[72]. Furthermore, computer-assisted image analysis provides a standardized and reproducible approach, reducing interobserver variability and enhancing the consistency and reliability of OSSN assessment[73].

Establishing deep learning frameworks for OSSN

Several deep learning frameworks have emerged that can facilitate the development and deployment of deep neural networks in OSSN research and clinical practice. For example, frameworks such as TensorFlow, PyTorch, and Keras offer a wide range of pre-built modules, functions, and tools that streamline the implementation of deep learning models, which provide a user-friendly interface and enable efficient model training and evaluation, allowing researchers and clinicians to explore new algorithms, validate research findings, and develop innovative solutions to improve the diagnosis and management of OSSN[74-76].

Validation of AI against expert panel

Validation of AI against expert panels is a crucial step in assessing the performance and reliability of AI algorithms in OSSN. The expert panel could consist of ophthalmologists, pathologists, and other specialists who have extensive knowledge and expertise in diagnosing and managing OSSN. By comparing the AI-generated assessments with the consensus decisions of the expert panel, the accuracy, sensitivity, specificity, positive predictive value and negative predictive value of the AI algorithm can be evaluated[5,77]. The validation process also highlights areas where the algorithm may outperform or fall short compared to human experts. This iterative process of validation and refinement is essential for optimizing AI algorithms, improving their accuracy, and gaining confidence in their reliability for clinical applications in OSSN[78].

Adoption of AI for OSSN

Need for shift in protocols: There is a growing need for a shift in protocols regarding the incorporation of AI algorithms in OSSN management. Traditional protocols often rely on manual evaluation and subjective interpretation by healthcare professionals, which can be time-consuming and prone to interobserver variability[79]. The integration of AI can streamline and enhance these protocols by providing objective, consistent, and efficient analysis of OSSN lesions. By leveraging AI's capabilities in image analysis, pattern recognition, and predictive modelling, protocols can be modified to include automated screening, diagnosis, classification, severity evaluation, and treatment prediction[14].

Furthermore, the implementation of AI in OSSN protocols can lead to improved efficiency and resource allocation due to faster screening, reducing patient wait times and optimizing clinical workflow. Moreover, identifying high-risk cases that require more immediate attention allows for timely interventions[80]. By integrating AI into protocols, healthcare providers can allocate their expertise and resources more effectively, focusing on complex cases and providing personalized care while AI handles routine tasks. Thus, it has the potential to enhance the overall efficiency, quality, and accessibility of OSSN management.

Phase of testing compliance of computer based image analysis: This phase involves ensuring that the AI algorithms and systems meet regulatory standards and compliance requirements. This phase focuses on evaluating their safety, reliability, accuracy, precision, robustness, and generalizability[81]. It also involves assessing the security and privacy aspects. Compliance testing aims to mitigate risks, validate the effectiveness of the technology, and gain regulatory approvals or certifications necessary for its use in clinical settings[82].

During compliance testing, the AI algorithms and systems are subjected to various validation and verification processes involving comparison of AI-generated results with ground truth data, conducting performance evaluations, and assessing the system's ability to handle different scenarios and variations[83]. Compliance testing also includes conducting comprehensive risk assessments to identify and address potential vulnerabilities or biases in the AI algorithms. Additionally, the systems' compliance with privacy regulations, such as data anonymization and encryption, is evaluated to ensure the protection of patient confidentiality.

Phase of initial acceptance: During this phase, healthcare professionals and institutions begin to integrate AI algorithms and systems into their workflows and protocols. The initial acceptance phase focuses on user acceptance, training, and education to ensure the successful implementation and utilization of the AI technology. Healthcare providers need to be familiarized with the capabilities, limitations, and benefits of the AI systems, as well as their integration into existing clinical processes[84]. Training may include workshops, demonstrations, and hands-on sessions to educate users. Additionally, clear guidelines and protocols are established to outline the roles and responsibilities of healthcare professionals when using AI systems, ensuring proper integration into clinical practice. Continuous support, feedback mechanisms, and collaborative efforts between AI developers and healthcare providers are crucial during this phase to address any challenges or concerns and facilitate a smooth transition. This phase marks the beginning of the widespread adoption of computer-based image analysis in OSSN, bringing the benefits of AI technology to the forefront of clinical decision-making and patient care[85].

Phase of full utilization: The phase of full utilization represents the culmination of the integration of computer-based image analysis in OSSN management. During this, AI technology will be fully embraced and incorporated into routine clinical practice for OSSN. Its utilization will become an integral part of the standard workflow, diagnosis and management, reflecting the optimization of processes and outcomes through the effective utilization of AI technology[86].

In the phase of full utilization, healthcare providers would have gained extensive experience and expertise in working with the AI systems. They would have developed a deep understanding of the technology's capabilities, limitations, and potential applications. Refinements would be based on user feedback, continuous learning, and advancements in the field through continuous quality improvement initiatives. Full utilization of computer-based image analysis will enable healthcare providers to leverage the benefits of AI for OSSN management, such as improved accuracy, efficiency, and standardization, leading to enhanced patient care, better treatment outcomes, and more effective resource allocation (Table 4)[87].

Table 4 Various advantages of integrated artificial intelligence in ocular surface squamous neoplasia

Advantages of integrated AI in OSSN
AI algorithms would analyze large volumes of data with speed and accuracy, surpassing human capabilities in terms of processing efficiency
This capability would allow for rapid and efficient screening, diagnosis, and evaluation of OSSN lesions, saving valuable time and resources for healthcare professionals
AI models would progressively learn from vast datasets, enabling them to identify increasingly complex patterns and subtle features that may be challenging for human observers to detect
This ability would offer enhanced diagnostic accuracy and aid in early detection, potentially improving patient outcomes and prognosis
AI would provide a standardized and objective assessment, reducing interobserver variability and ensuring consistent and reliable evaluations of severity, classification, and staging
By leveraging AI technology, clinicians would benefit from enhanced decision support, optimized treatment planning, and personalized management strategies for OSSN patients

AI: Artificial intelligence; OSSN: Ocular surface squamous neoplasia.

CONCLUSION

The future of deep learning-based image analysis in OSSN holds immense potential for further advancements and transformative impacts. With the availability of larger and more comprehensive datasets, deep learning models can be trained to extract even more nuanced features and patterns from OSSN images, leading to improved diagnostic accuracy and precision.

Additionally, the integration of multimodal data, such as combining imaging information with molecular profiling or clinical data, can enable more precise risk stratification, treatment prediction, and personalized management approaches. Furthermore, the utilization of real-time data and the incorporation of dynamic imaging techniques, such as video-based analysis or sequential image analysis, can provide valuable insights into the temporal changes and progression of OSSN, supporting more proactive and timely interventions[88].

AI algorithms can be leveraged for treatment planning, monitoring treatment response, and predicting long-term outcomes. By integrating deep learning with clinical and genetic data, personalized treatment plans can be developed, optimizing therapeutic strategies and improving patient outcomes. Moreover, the development of user-friendly interfaces, integration with electronic health records, and advancements in telemedicine technologies can facilitate the widespread adoption and utilization of deep learning-based image analysis in OSSN, allowing for seamless integration into routine clinical practice and improving access to specialized care.

Overall, the future of deep learning-based image analysis in OSSN holds great promise in revolutionizing the field, enabling more accurate diagnoses, personalized treatment approaches, and improved patient care.

FOOTNOTES

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Country/Territory of origin: India

ORCID number: Sony Sinha 0000-0002-6133-5977; Prasanna Venkatesh Ramesh 0000-0002-6105-8666; Prateek Nishant 0000-0003-3438-0040; Arvind Kumar Morya 0000-0003-0462-119X.

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MINIREVIEWS

Did pediatric drug development advance epilepsy treatment in young patients? It is time for new research goals

Earl B Ettienne, Emilio Russo, Pasquale Striano, Jane M Grant-Kels, Klaus Rose

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Earl B Ettienne, College of Pharmacy, Howard University College of Pharmacy, Washington, DC 20059, United States

Emilio Russo, Department of Health Sciences, School of Medicine, Russo, University "Magna Graecia" of Catanzaro, Catanzaro 88100, Italy

Pasquale Striano, IRCCS Istituto Giannina Gaslini, Genova 16148, Italy

Jane M Grant-Kels, Department of Dermatology, University of Connecticut Health Center, Farmington, CT 06032, United States

Klaus Rose, klausrose Consulting, Pediatric Drug Development and more, Medical Science, CH-4125 Riehen, Switzerland

Corresponding author: Klaus Rose, MD, MS, CEO, klausrose Consulting, Pediatric Drug Development and more, Medical Science, Äussere Baselstrasse 308, CH-4125 Riehen, Switzerland. klaus.rose@klausrose.net

Abstract

Modern drugs have changed epilepsy, which affects people of all ages. However, for young people with epilepsy, the framework of drug development has stalled. In the wake of the thalidomide catastrophe, the misconception emerged that for people < 18 years of age drugs, including antiseizure medications (ASMs), need separate proof of efficacy and safety, overall called "pediatric drug development". For ASMs, this has changed to some degree. Authorities now accept that ASMs are effective in < 18 years as well, but they still require "extrapolation of efficacy," as if minors were another species. As a result, some of the pediatric clinical epilepsy research over the past decades was unnecessary. Even more importantly, this has hampered research on meaningful research goals. We do not need to confirm that ASMs work before as they do after the 18th birthday. Instead, we need to learn how to prevent brain damage in young patients by preventing seizures and optimize ASMs' uses. Herein we discuss how to proceed in this endeavor.

Key Words: Epilepsy; Pediatric drug development; Therapeutic orphans; Antiseizure medications; Pediatric investigation plan; Clinical pharmacology

Core Tip: For young people with epilepsy, the framework of drug development has stalled. The misconception emerged that for people < 18 years drugs, including antiseizure medications (ASMs), need separate proof of efficacy and safety, overall called "pediatric drug development". For ASMs, the authorities require "extrapolation of efficacy", as if minors were another species. Relevant parts of pediatric epilepsy research were pointless, and research on meaningful goals was hampered. ASMs work also before the 18th birthday. We should learn to prevent brain damage in young patients by preventing seizures and by optimize ASMs' use.

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INTRODUCTION

Antiseizure medications (ASMs) have changed epilepsy from the dramatic connotation of "falling sickness" in the past to a chronic condition that can often be prevented and managed. ASMs prevent seizures in many, but unfortunately not all patients[1,2]. In the United States, about 1.2% of the total population had active epilepsy in 2015, corresponding to 3.4 million persons, *i.e.* 3 million adults and 470000 minors. About 0.6% of persons aged 0-17 years had active epilepsy, *i.e.* about 6 out of every thousand minors[3]. Epileptic seizures affect patients of all ages. Most seizures resolve on their own, but the younger a child is at diagnosis, the higher the danger that frequent seizures or status epilepticus (SE) will cause lasting brain damage; furthermore, disease progression is not limited to more seizures[4-6]. Even if we can only prevent brain damage in a small percentage of young patients, that would be worth the effort.

A century ago there was no ASM, so drug development has been excellent. However, it is counterproductive that there is a demand for additional approval of all medications for chronologically defined children, including ASMs. This has resulted in recent decades in numerous clinical studies to prove again efficacy and safety of ASMs in legally and chronologically defined minors, as if they represent a different species. Epileptology was the first academic clinical discipline to distance itself from this basic assumption[7-9]. However, several major challenges remain. First, the awareness that research efforts during the last decades could have been used much better in the treatment of epilepsy in minors is not widespread in the clinical world. Second, there is a lack of awareness that this is a challenge far beyond epilepsy. Third, the waste of research energy over the last decades was based on massive conflicts of interests, the addressing of which has until now been largely taboo. In our opinion, this represents a fundamental challenge to modern science and medicine.

Over the last decades, the pharmaceutical industry's alleged greed served as a welcome scapegoat to force companies to carry out supposedly "good" clinical studies in minors. Sadly, the reality was different and included: (1) The combination of a society-wide illusion in the clinical value of separate drug approval in minors; (2) the desire for funds, careers, and reputation of some academics, and their supporters in the regulatory authorities; and (3) the Western academic world accepted an unfortunate social issue.

Acceptance while not paying attention to potential warning signs and dangers in science or political events can be perilous. When Russia developed the first anti-corona virus infectious disease 2019 (COVID-19) vaccine and tried to use it to gain diplomatic advantages and greater prestige around the world[10], few were paying attention that Russia was also preparing the invasion of Ukraine. COVID-19 was a medical challenge, while the invasion of Ukraine was a military aggression. Both were carried out by the same government. It was much more convenient to ignore warning. In our view, ignoring the conflicts of interest behind "pediatric drug development" (PDD) and resulting pointless ASM studies in minors is a similar scenario.

We analyzed characteristics and impact of separate pediatric clinical studies on ASMs with the background of the history of United States and European Union (EU) pediatric legislation, which resulted in "PDD"[11-13]. On this basis, we describe how the PDD requirements for ASMs evolved from the 1990s to today; how academic criticism has changed the demand profile of the regulatory requirements and how, in hindsight, many pediatric epilepsy studies should be classified as pointless. We conclude that excessive demands for "pediatric" ASM studies should be replaced by meaningful studies with the potential to improve the care of pediatric epilepsy patients.

ASMS, PDD, AND EVIDENCE-BASED MEDICINE

Drug approval is today based on regulatory clinical and other studies within an elaborate bureaucratic process. The United States introduced the obligation to prove the efficacy and safety of new drugs in 1962 as a response to the thalidomide disaster. The resulting procedure has become standard worldwide in the following decades[14]. This framework requires, inter alia, a functional public administration, social trust, and a societal balance of influence of the classical healthcare professions, drug manufacturers, regulatory authorities, public opinion, patients, and for underage patients' caregivers/parents. Two additional factors have in the last decades come into play for young patients that need to be considered when reflecting about future epilepsy research. (1) PDD assumes that in chronologically defined

"children" separate drug approval is needed, resulting in separate "pediatric" labels. The requirement for PDD is set out in United States and EU laws[11-17]. "Children" and "pediatric" are in quotation marks because the globally used age limit of < 18 years does not even come close to the end of puberty when the child's body becomes mature. The process of puberty has accelerated over the past 100 years[18,19]. Clinical studies in adolescents are physiologically and scientifically not pediatric studies, but the regulatory authorities are maintaining this illusion. This is of specific relevance for epilepsy in young patients as epilepsy was the first broad clinical area where the Food and Drug Administration (FDA) and European Medicines Agency (EMA) halfway accepted that drugs work after and before the 18th birthday[20-22], triggered by critical review of "pediatric" research by academic neurologists[7-9]. However, even the term "extrapolation of efficacy" from adults to minors is misleading. Minors are not another species[14-16]. The PDD concept is best expressed by the mantra of children as "therapeutic orphans"[23], taken up by the American Academy of Pediatrics and the historically new discipline of developmental pharmacology[11,12,14-16]; and (2) the concept of evidence-based medicine (EBM). From 1962 onwards, in the wake of the United States processing of the thalidomide disaster and the resulting modification of pharmaceutical law, randomized clinical trials to prove drug's efficacy and safety became widespread [17].

Based on the awareness of the limitations of traditional determinants of clinical decisions, EBM became in the view of clinicians a new paradigm. EBM was a step forward in replacing traditional personal authority by the dictate of independently produced data[24-26]. EBM with young people defines a group of people according to chronological, not physiological criteria. These chronological criteria are administrative in nature, not scientifically based. Clinicians may overlook that most large clinical trials were and are registration studies for new drugs, not simply independent data. An exception was pediatric oncology, where chemotherapeutic agents had already existed for decades but were systematically tested for malignancies in minors only since the 1960s[14,27]. These studies and the resulting treatment protocols resulted in saving the lives of hundreds of thousands of minors, but not in drug labels, which representatives of the EMA criticized seriously[28].

The 18th birthday is an administrative age limit and does not correspond to a physiological change. Drugs treat the body, not the legal status. Amplifying the original United States PDD approach, EU law and the EMA demand a "pediatric investigation plan" (PIP) for all new drugs[13], except those that target a disease the EMA recognizes as non-existing in "children". Companies have to commit to "pediatric" studies, resulting in a repetition of clinical development in minors < 18 years, subdivided into the subgroups defined by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use: Preterm neonates; neonates; infants and toddlers; children; and adolescents[29]. EU-PIP-demanded studies recruit worldwide.

In 1994 the United States National Institute of Health organized a conference on drug treatment of children with epilepsy as part of the emerging PDD movement. Clinicians then used ASMs in minors irrespective of separate "pediatric" approval. Nevertheless, the regulators now wanted separate proof of efficacy and safety also in "children". The non-government participants opined that drug efficacy for focal seizures in adults justified their approval in young patients provided safety and pharmacokinetic were proven, an appropriate and "common sense" approach in contrast to the strict regulatory approach that has dominated the next 30 years and which we consider "dogmatic". Sheridan *et al*[30] did not address the difference between the physiological and legal/administrative meanings of the term "child"[30,31].

DOGMATIC REGULATORY PDD APPROACH

Since 2019 the FDA accepts extrapolation of efficacy for focal-onset seizure (FOS) epilepsy in young patients down to two years[20], justified by advances in understanding disease pathophysiology and progression in epilepsy research[21]. In its official justification, the FDA uses what is considered by some as an outdated term "partial onset seizure (POS)"[20]. The EMA accepts this extrapolation at present down to 4 years. A review of all studies from 1970-2010 on adjunctive therapy in FOS in adults and children concluded that ASMs are effective for FOS in children as well, once efficacy is proven in adults[7-9]. Under 2 years there were not enough patients and trials. In our view, the term "extrapolation" of the efficacy of ASMs from adults to "children" is misleading. "Extrapolation" implies a fundamental difference between adults and "children". There are real differences between infants and adults, but they are the same species. Furthermore, children grow and do not remain newborn and vulnerable until they reach the official age of maturity.

Extrapolating observed toxicities in preterm newborns to all "children" (and silently switching the use of the term "child" from its physiological to its legal/administrative meaning), the advocates of PDD managed to convince lawmakers that separate "pediatric" labels would resolve a major healthcare crisis and would allow a fundamental step forward in pediatric healthcare[32]. What clinicians had advised in 1994 was decades later accepted by the FDA and eventually also the EMA: Regarding ASMs, it is legitimate to call "pediatric" patients small adults: The underlying pathophysiological process is the same in all patients > 2 years. Young patients will respond similarly to ASMs, provided an equivalent serum concentration-time profile. Neurology was one of the first clinical disciplines to openly challenge the children-are-therapeutic-orphans concept[31]. However, it is not sufficient to "just" accept that ASMs are effective before and after the 18th birthday. It is necessary to candidly address the misconception that separate labels in patients < 18 years have any substantial clinical benefit. The formulas and dosing tables used by pediatricians and family doctors in the 1960s were largely sufficient, except for preterm. In our opinion, the PDD movement was a regulatory exercise with much official success but no real clinical progress.

Several other clinical areas face the same dilemma. There is no such thing as separate "pediatric" and "adult" rheumatoid arthritis[33]. The conditions summarized under "juvenile idiopathic arthritis" (JIA) are disorders that begin early and persist into adulthood[34-37]. They were first described when diseases in young people were systematically

studied in the second half of the 20th century, including childhood cancer, childhood joint inflammation, childhood skin inflammation, and the like. It was thought at the time that these were separate "pediatric" diseases. They had not been observed before because most of these diseases are so rare that an individual family doctor may see them once or twice in their entire professional life (and there was no internet in these times). Patients with systemic JIA (sJIA) diagnosed early do not switch to classical rheumatoid arthritis later. Only the patient's administrative status changes. Conventional malignant melanoma in adolescents and adults is the same disease and needs the same treatment[38,39]. There are extremely rare forms of a distinctive variant of melanoma that is diagnosed in very young children and infants, not adolescents.

Another good example from neurology is amyotrophic lateral sclerosis (ALS), a progressive motor neuron disease, leading to progressive paralysis, and eventually to death. Most cases are sporadic, but some are familial with a wide variety of genetic mutations. No definitive diagnostic test or biomarker exists so far. The diagnosis is made by clinical observations. The peak age at onset is 58–63 years for sporadic and 47–52 years for familial disease. From the age of 80 years on the incidence decreases rapidly. Symptomatic multidisciplinary treatment can slow down disease progression [40]. In a small subgroup of patients, onset is described as < 25 years; in even rarer cases, symptoms start before the 18th birthday. A literature research found twenty-nine such pediatric cases reported in the literature[41], while a European survey identified thirty-nine such patients[42]. The literature discusses both juvenile ALS, starting < 25 years, and pediatric ALS, even more rare, beginning < 18 years of age[43]. The more genetic mutations that are identified, the more ALS in young patients appears to be less a uniform disease and more an overlapping of degenerative motor neuron deficits based on different genetic mutations. After the EU pediatric regulation had been enacted, the EMA had issued a "waiver" for various predominantly adult diseases, including malignant melanoma, ALS, and more, meaning that no PIPs were needed. But in 2015, the EMA declared that from now on it classified several predominantly adult diseases also as "pediatric", including ALS, liver carcinoma, kidney carcinoma, Parkinson disease, Huntingdon chorea, and more[44]. For ALS it claims that there is no criterion other than age to distinguish juvenile from adult onset ALS forms, referring to three publications[45-47]. However, these publications do not support the EMA's claims. Orban *et al*[45] describe familial neurodegenerative diseases based on various genetic mutations. The paper's title is "Juvenile ALS", but the syndromes also have various other names. Juvenile onset ALS is defined as beginning < 25 years[45]. Finsterer *et al*[46] discuss the genetics of motor neuron diseases. Several genetic mutations are found in syndromes described as "juvenile" beginning at < 20 years of age[46]. Bertini describes juvenile ALS as a very rare severe motor neuron disease beginning at < 25 years of age. Bertini 2014 is not peer-reviewed, but a 1-page orphanet entry by a single author[47].

In 2020, 58 ALS clinical experts affiliated with the European Network to Cure ALS[48], participated in a survey on PIP-demanded pediatric ALS studies. All questions had been reviewed by the executive board of Treatment Research Initiative to Cure ALS[49], comprising neurologists from eight European countries. Thus, the survey represents the best clinical ALS knowledge in Europe. It concluded that pediatric ALS as a disease entity does not exist, patients < 18 years are extremely rare, and that the pediatric studies demanded by the EMA and its Pediatric Committee (PDCO) would be unfeasible and a waste of money and resources[42].

Thus, in several clinical areas the regulatory claim of two forms of the same disease, an adult one (> 18 year) *vs* a "pediatric" one (< 18 year) is no longer supported.

THE DOGMATIC REGULATORY APPROACH TO "CHILDREN" IS FAR FROM OVER

For heads of medical departments, raising funds for clinical research is a key responsibility. Industry-paid funds triggered by regulatory demands were and are a temptation. The number of ongoing "pediatric" studies has diminished, but clinicians have not yet processed sufficiently the fundaments of the temptations of "pediatric" clinical studies.

The flawed belief that the 18th birthday corresponds to a physiological change has resulted in superfluous clinical studies in adolescents and studies in younger minors, where dose-finding would have been clinically required. The main conclusions by Pellock, Arzimanoglou *et al*[8,9] were eventually accepted by FDA/EMA, but large international clinical studies in "children" that often were physiologically no longer children continued worldwide. Also the formal–and flawed-distinction between adult and "pediatric" patients continues. The division of the medical world into "pediatric" and "adult" disciplines is administrative, not scientific. Whether a 15-year-old casualty is placed in a pediatric or adult surgical ward is medically irrelevant. It is an administrative question. But the same disease before or after the 16th, 17th or 18th birthday should neither have different names nor should be treated differently. This is the case when there is a pediatric and an adult rheumatology department in the same hospital, and where adult and "pediatric" rheumatology use different names for the identical disease, *e.g.* sJIA[37].

A NECESSARY PARADIGM SHIFT

"Pediatric" ASM drug studies were performed worldwide in up to 118 medical centers[14-16,50-52]. Parents have become reluctant to allow the participation of their loved ones in "pediatric" epilepsy studies[53]. The studies demanded by regulatory authorities would need more "pediatric" patients than exist worldwide[54]. The EMA knows this, the PIPs continue, and FDA and EMA continue to publish common statements that explain "pediatric" requirements even for vaccines and drugs against COVID-19[55].

Well-intended but flawed regulatory requirements in the name of EBM, PDD and "better medicines for children" led to many pointless studies and publications. The EMA has issued PIPs for most modern ASMs, demanding studies in "children" < 18 years. Some PIPs have translated into studies, others are in various stages of preparation, many are ongoing, and some PIPs have silently been converted into waivers, *i.e.* no "pediatric" studies are required any more[14-16]. The EMA continues to call PDD "better medicines for children"[56]. In our view, the current focus of pediatric epilepsy research is too focused on drug approval in minors. Such studies require complex and expensive logistics but do not improve treatment. Instead, we propose a shift away from clinically pointless, but formally regulatorily justified "pediatric" studies to clinically meaningful studies. Such studies will not prove again that ASMs work before and after the 18th birthday. Instead, we propose to simply accept their efficiency and instead explore how they can be better used.

In young children, self-management means the administration of emergency ASMs by parents/caregivers. Patel *et al* [57] initiated a regional project to involve parents. This reduced the number of SE emergency admissions by 28%[58]. A paper on acute seizure action plans compares the self-management guides for patients and caregivers in asthma and diabetes to SE. Stredny *et al*[59] emphasize infrequent or restricted use of home rescue medications by caregivers[59]. Buchhalter aims at recommendations from a broad stakeholder group including caregivers[60]. The pediatric SE research group investigated the timing and escalation of ASM administration in pediatric SE both in the prehospital and in-hospital settings[61,62]. Training of caregivers is addressed by several authors[4,63,64].

A further dimension is the patient's quality of life (QoL). Today, seizure control is considered the primary goal but when a patient is treated with many drugs, other QoL issues are impacted, including weight gain or altered mentation. Parents, doctors and older patients should decide together on balancing priorities. This might include the acceptance of occasional seizures in return for a better overall QoL. Better integrating parents into the prospective care of children at risk for epileptic seizures or those who have already had seizures is recommended[65]. Hopefully, parents' advocative groups for Dravet syndrome, Lennox Gastaut Syndrome, Sturge Weber Syndrome, and more will contribute to funds, coordination, and intellectual processing of such a new strategy. In 1994, reimbursement of drugs not approved for "children" was not a problem[30]. Today, administrative aspects have acquired a stronger role in medical care. Nonetheless, the lack of "pediatric" approval is no valid clinical justification for separate studies.

A CHALLENGE BEYOND EPILEPSY

Overall, this is a gigantic challenge for the next decades. Only since the public administration issued identity documents for all is the precise date of birth immediately available in an officially certified form, and hence the conceptual creation of a chronologically and administratively defined "pediatric population" [66]. The 20th century also brought the discovery and industrial production of effective drugs. The triangle of (1) Initially chemical, then pharmaceutical, and now life sciences industry; (2) academia and clinical care; and (3) regulatory authorities emerged as a framework for drug development. With the globalization of economy and drug development, an additional international framework emerged, operating alongside and above national drug approval. All institutions of public administration became stronger and developed their own self-interests.

Drug development, public administration and the new discipline of developmental pharmacology met the new institution of regulatory authorities and increased public interest in children's well-being. When it was recognized that absorption, distribution, metabolism, excretion (ADME) of drugs in premature infants is different from ADME in older people, this developed into the children-are-not-small-adults and children-are-therapeutic-orphans mantras, reflected in the PDD legal codes. Using the history of "pediatric" epilepsy studies, we traced how the demand for separate approval of ASMs in "children" emerged, "children" defined legally/administratively, not scientifically and physiologically. It took almost half a century until Pellock *et al*[7] questioned the legitimacy of these pediatric efficacy and safety studies[7-9,31]. Unforunately, the first systematic criticism of PDD, first in peer-reviewed journals and then in medical textbooks, only began in 2014[14-16,39,67].

Epilepsy is investigated and treated in the context of neurology. The challenge goes far beyond neurology. It is a challenge for humanity in the 21st century to distinguish in medicine between the legal and administrative definition of minors and the physiological definition of premature infants and children.

The EMA demands "pediatric" studies on underage mothers with postpartum depression and on underage athletes with cartilage injuries in the knee joint between the physiological end of puberty, as evidenced by the closure of the growth plates, and the 18th birthday[14-16]. Such studies are scientifically and medically unnecessary. We identify the extent of the abuse of young people in clinical research only when we look beyond individual medical disciplines. All the discussed "pediatric" studies are in direct violation of the Declaration of Helsinki[68]. They are a blatant abuse of power, based on conflicts of interest that are not captured by the control mechanisms currently in use[69].

Medicine has made great progress in the last decades and centuries. But it has also become a comfortable professional field in which individual young participants compete to rise in the hierarchy of power and influence. The origin of PDD lies in developmental pharmacology, the emergence of organized pediatrics, and the contemporary emergence of regulatory authorities. We are of the opinion that it is a task for every neurologist to check which questionable "pediatric" studies are being carried out at their workplace. In distinguishing between "pediatric" and adult research in neurology and other medical disciplines, administrative matters must be separated from those that are truly medically justified. This is a task not only for pediatric neurology, but for the entire medical profession.

FUTURE PEDIATRIC EPILEPSY RESEARCH

The avenue of the future should not be to ask for additional and larger, multicenter, pediatric-specific randomized clinical trials, nor more standardized diagnostic and treatment algorithms along with prospective multicenter studies; nonetheless. Such studies and research targets continue to be advocated[64,70-72].

Regulatory authorities support the narrative that only on-label drugs are safe for children and adults[73]. However, the entire discipline of pediatrics with the sub-disciplines of pediatric neurology, oncology, and neonatology emerged decades before the term "off-label" emerged in 1988[74]. There is a respect for the regulatory authorities as they have an important role in drug approval, but they are not scientific authorities.

A recent paper reviews the current international literature on management protocols for SE in the pediatric emergency room[75]. In our view, it would be desirable that fewer children with SE reach the pediatric emergency room if their caregivers were able to intervene earlier[4,64,76,77]. Self-management of epilepsy has in the last years been discussed, but in contrast to asthma or diabetes mellitus, it is still in its infancy[58,78,79].

REMAINING OPEN QUESTIONS

Pellock *et al*[7,8] accepted the age limit of two years for pragmatic reasons[7-9]. Novotny *et al*[50] confirmed that adjunctive topiramate does not reduce the daily rates of FOS in infants aged 1-24 months[50]. What is the true age limit? Parents might be too enthusiastic to administer emergency medication. There will never be a perfect scheme regarding decisions on the balance of QoL *vs* intake of multiple drugs. For such discussions and decisions, EBM does not provide any valid guidance.

DISCUSSION

Table 1 summarizes our overview over historical events. It shows how the clinical development of all new drugs since 1962 has taken place in an interplay between drug developers, now mostly industrial companies; clinicians; and the regulatory authorities. With the concept of PDD, the FDA began to actively intervene in drug development, which was continued and expanded by the EU and its EMA. The concept that children are completely different from adults led to the requirement of separate pediatric efficacy and safety studies despite the fact that the regulatory authorities define children administratively, *i.e.* chronologically, not scientifically and/or physiologically. On the night of the 18th birthday, the body does not fundamentally change even though the legal status does. The clinical analyzes from Pellock *et al*[8] in 2012 onwards showed that, at least in POS epilepsy, medications that work after the 18th birthday also work before that birthday[8].

The hands-on running of clinical regulatory studies is done by clinicians, albeit not in a vacuum. The clinicians overestimate their own role and underestimate the two other sides in the triangle in which drug development has taken place since 1962: The life science companies and the regulatory authorities. The concept that the 18th birthday corresponds to a physiological change is maintained, reflected in the expression "extrapolation". Does extrapolation from an 18 years old adult to a 17 years old teenager make sense? No. Are they fundamentally different from each other? Medically no, but administratively yes. In 2019 the FDA accepted that ASMs work also before the 18th birthday but used the term "extrapolation of efficacy"[80]. The EMA continues to defend "extrapolation of efficacy" for ASMs, equating approval of ASMs before the 18th birthday with their alleged "availability", as if they would not exist and could not be used before the 18th birthday[81]. Now, at least, the EMA accepts extrapolation from adults to young patients[82].

CONCLUSIONS

Regarding PDD, we refer to a recent publication in which authors representing regulatory authorities from around the world describe PDD as an ecosystem[83], which we regard as a Freudian slip. Ecosystems have inflows and outflows. The inflow of the PDD ecosystem is the payments from the life sciences industry. The outflow of PDD studies are "pediatric" labels, careers in "pediatric" research, "pediatric" publications, and a further expansion of academic and clinical research activism that has become, to a large extent, a self-purpose, sold to the general public, politicians, and young researchers early in their career as an essential path to scientific and medical progress and better care for children.

Medicine in the 21st century will have to learn that neither the regulatory authorities nor the World Health Organization are white knights who represent nothing but the truth and the interests of patients. Our world has become more complex and conflicts of interest are increasingly hidden beneath the surface. Science has had to overcome other major obstacles in the past. The consequence for the individual disciplines will be that they will have to learn to think outside the box again. The conduct of purportedly pediatric clinical trials is overall the greatest abuse of patients in the history of medicine, going well beyond the classic, often-cited examples of the published studies criticized by Beecher in 1966 and the Tuskegee study terminated in 1972[84,85]. Conferences of all major medical disciplines will have to address and discuss this issue.

Epilepsy research will hopefully direct itself to new, truly innovative goals. Self-management of diseases is not a radically new concept. Herein, we have put our thoughts into the context of flawed demands for ASMs efficacy and

Table 1 Key events in the tension between pediatric drug development and epilepsy clinical research

Ref.	Year	Event
[23]	1950	Toxicities are reported in preterm newborns treated with antibiotics
[17]	1962	United States pharmaceutical legislation (Keifauver-Harris Amendment)
[23]	1968	Shirkey coins the term "therapeutic orphans"
[8, 30]	1996	United States National Institutes of Health "pediatric" antiseizure medications workshop in Bethesda, MD, United States
[32]	1997	United States pediatric legislation
[13]	2006	European Union pediatric legislation
[80]	2010	European Medicines claims fundamental difference in pediatric *vs* adult epilepsy, demands studies in > 100 children for new drugs
[7]	2012	Efficacy of antiseizure medications in adults predicts efficacy in minors
[22]	2016	Proposal for a new paradigm
[8]	2017	Proof that antiseizure medications work both in adults and minors
[11]	2018	Justification of "pediatric drug development" repeated
[20]	2019	Food and Drug Administration accepts extrapolation for antiseizure medications adults to minors
[80]	2019	European Medicines authors equate antiseizure medications approval with "availability," as if without approval they would not exist for patients before their 18th birthday
[81]	2023	EMA accepts extrapolation from adults to "children"

safety studies in "children". Involving and training parents will have many new challenges. In our view, our conclusions are to some degree obvious: ASMs work in young person equally well before and after the 18th birthday. The time has come to candidly address alleged pediatric research in neurology and beyond that is not really pediatric; to suspend ongoing questionable trials; to reject questionable studies newly submitted to Institutional Review Boards/ethics committees; and to focus on ways to better engage caregivers and parents.

FOOTNOTES

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Country/Territory of origin: Switzerland

ORCID number: Earl B Ettienne 0000-0002-5859-3086; Emilio Russo 0000-0002-1279-8123; Pasquale Striano 0000-0002-6065-1476; Jane M Grant-Kels 0000-0002-1844-8415; Klaus Rose 0000-0002-8304-1822.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Tracheostomy-related data from an intensive care unit for two consecutive years before the COVID-19 pandemic

Maria Papaioannou, Evdoxia Vagiana, Serafeim-Chrysovalantis Kotoulas, Maria Sileli, Katerina Manika, Alexandros Tsantos, Nikolaos Kapravelos

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Maria Papaioannou, 1st Intensive Care Unit, G Papanikolaou General Hospital, Exohi, Thessaloniki 57010, Greece

Evdoxia Vagiana, Maria Sileli, Nikolaos Kapravelos, 2nd Intensive Care Unit, G Papanikolaou General Hospital, Exohi, Thessaloniki 57010, Greece

Serafeim-Chrysovalantis Kotoulas, Intensive Care Unit, Hippokration General Hospital, Thessaloniki 54642, Greece

Katerina Manika, Department of Pulmonary, Medical School, Aristotle University of Thessaloniki, G. Papanikolaou General Hospital, Exohi, Thessaloniki 57010, Greece

Alexandros Tsantos, 2nd Department of Internal Medicine, General Hospital of Thessaloniki "Ippokration", Thessaloniki 54642, Greece

Corresponding author: Serafeim-Chrysovalantis Kotoulas, PhD, Consultant Physician-Scientist, Intensive Care Unit, Hippokration General Hospital, Kostantinoupoleos 49, Thessaloniki 54642, Greece. akiskotoulas@hotmail.com

Abstract

BACKGROUND

Tracheostomy is commonly used in intensive care unit (ICU) patients who are expected to be on long-term mechanical ventilation or suffer from emergency upper airway obstruction. However, some studies have conflicting findings regarding the optimal technique and its timing and benefits.

AIM

To provide evidence of practice, characteristics, and outcome concerning tracheostomy in an ICU of a tertiary care hospital.

METHODS

This was a retrospective cohort study including adult critical care patients in a single ICU for two consecutive years. Patients' demographic characteristics, severity of illness (APACHE II score), level of consciousness [Glasgow Coma Scale (GCS)], comorbidities, timing and type of tracheostomy procedure performed and outcome were recorded. We defined late as tracheostomy placement after 8 days or no tracheotomy.

RESULTS
Data of 660 patients were analyzed (median age of 60 years), median APACHE II score of 19 and median GCS score of 12 at admission. Tracheostomy was performed in 115 patients, of whom 63 had early and 52 late procedures. Early tracheostomy was mainly executed in case of altered level of consciousness and severe critical illness polyneuromyopathy, however there were no significant statistical results (47.6% *vs* 36.5%, $P = 0.23$) and (23.8% *vs* 19.2%, $P = 0.55$) respectively. Regarding the method selected, early surgical tracheostomy (ST) was conducted in patients with maxillofacial injuries (50.0% *vs* 0.0%, $P = 0.033$), whereas late surgical tracheostomy was selected for patients with goiter (44.4% *vs* 0.0% $P = 0.033$). Patients with early tracheostomy spent significantly fewer days on mechanical ventilation (15.3 ± 8.5 *vs* 22.8 ± 9.6, $P < 0.001$) and in ICU in general (18.8 ± 9.1 *vs* 25.4 ± 11.5, $P < 0.001$). Percutaneous dilatation tracheostomy (PDT) *vs* ST was preferable in older critical care patients in the case of Central Nervous System underlying cause of admission (62.5% *vs* 26.3%, $P = 0.004$). ST was the method of choice in compromised airway (31.6%, *vs* 7.3% $P = 0.008$). A large proportion of patients (88/115) with tracheostomy managed to wean from mechanical ventilation and were transferred out of the ICU (100% *vs* 17.4%, $P < 0.001$).

CONCLUSION
PDT was performed more frequently in our cohort. This technique did not affect mechanical ventilation days, ventilator-associated pneumonia (VAP), ICU length of stay, or survival. No complications were observed in the percutaneous or surgical tracheostomy groups. Patients undergoing early tracheostomy benefited in terms of mechanical ventilation days and ICU length of stay but not of discharge status, presence of VAP, or survival.

Key Words: Tracheostomy; Early tracheostomy; Late tracheostomy; Percutaneous dilatation tracheostomy; Surgical tracheostomy; Weaning; Survival; Mechanical ventilation

Core Tip: Performing a tracheostomy in critical care patients is a common procedure. We analyzed patients who were hospitalized for two consecutive years in an intensive care unit in a tertiary hospital, before the coronavirus disease 2019 pandemic. We recorded our findings in this observational study, associated with the timing and method of tracheostomy, the role of tracheostomy in weaning from the mechanical ventilation and the outcome. Our findings were quite consistent with the review of literature, but need to be confirmed by prospective studies. We hope that this study could contribute to a certain degree to the literature about tracheostomy.

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INTRODUCTION

Tracheostomy is a long-established invasive intervention commonly performed in critically ill patients treated in the intensive care unit (ICU). It is primarily carried out to wean patients who may require prolonged invasive mechanical ventilatory support and in emergency upper airway obstruction[1]. Tracheostomy is a safe procedure when used on an elective basis, which has been shown to have benefits compared to prolonged translaryngeal ventilation. Some advantages include avoiding laryngeal injury, protecting against pulmonary aspiration, facilitating clearance of respiratory secretions, decreasing sedation needs, supporting nursing care, and enhancing patients' comfort and daily living activity[2]. Furthermore, there is increasing evidence that tracheostomy shortens the length of ICU stay and decreases the risk of developing ventilator-associated pneumonia (VAP)[3]. Well-known complications are bleeding, pneumothorax, stomal infection, and tracheal stenosis[4].

Two approaches are feasible: the open surgical tracheostomy (ST) and the bedside percutaneous dilatational tracheostomy (PDT) performed by intensive care physicians[1]. The choice depends on patient risk factors. Both techniques bear advantages as well as early and late complications, with the PDT being preferred mainly due to less procedural time, effort consumption, and costs[5]. Evidence on the optimal timing for tracheostomy is still conflicting. Previous meta-analyses showed no difference in the duration of mechanical ventilation, incidence of VAP, or short-term mortality[6,7]. A recent meta-analysis by Chorath *et al*[8] demonstrated lower VAP rates and shorter durations of mechanical ventilation and ICU stay.

Tracheostomy practices in ICUs vary between countries, even between different regions of the same country. Our study aimed to provide evidence of practices, characteristics, and outcomes concerning tracheostomy in the ICU of a tertiary care hospital.

MATERIALS AND METHODS

The protocol of this retrospective observational study was approved by the scientific council of the General Hospital of Thessaloniki "G. Papanikolaou," reference number 1214, and a relative from each participant gave written informed consent. Participants were all patients admitted to the 2nd Intensive Care Unit of the tertiary General Hospital of Thessaloniki "G. Papanikolaou" between 01/03/2018 and 31/08/2019, with no exclusion criteria. No sample size calculation was performed. Instead, it was decided to include all the patients for two consecutive years to better represent this specific unit's epidemiological data before the coronavirus disease-19 (COVID-19) pandemic. The same group of ICU physicians used the same PDT technique while the same team of surgeons performed the ST.

The variables that were recorded were the age and the gender of the patients, their Glasgow Coma Scale (GCS)[9] and their acute physiology and chronic health evaluation (APACHE II) and APACHE II predicted death rate (APDR)[10] at admission, their number and category of co-morbidities, their cause of ICU admission and intubation [central nervous system (CNS), cardiovascular system (CVS), respiratory system, sepsis, malignancy, trauma, surgery, metabolic disease compromised airway]. Furthermore, the performance of a tracheostomy, the reason why a tracheostomy was performed (prolonged mechanical ventilation, compromised airway, low level of consciousness, myopathy), the timing of tracheostomy (early *vs* late)[11], the reason for an early tracheostomy (prolonged duration of stay, compromised airway, trauma, neuromuscular disease), the method of the performed tracheostomy (PDT *vs* ST)[12], the reason for a ST (facial trauma, cervical burn, difficult airway, cervical edema, goiter, re-opening), any serious complications during the procedure, the occurrence of VAP, the responsible pathogen for the VAP, isolated by a bronchial secretion culture and variables related with the outcome of the patients, such as where they were transferred after their ICU discharged [ward, another hospital, rehabilitation center, critical care unit (CCU), or intensive coronary care unit (ICCU)], their breathing style at discharge (unassisted or assisted) and more specific their method of breathing (T-piece, speaking valve, tracheostomy closure, continuous positive airway pressure (CPAP), bi-level positive airway pressure, pressure or volume ventilator), their days spent on mechanical ventilation and in ICU and their weaning from mechanical ventilation and survival status.

Analysis was carried out using SPSS Statistics 24.0 software (IBM Corp, Armonk, NY, United States). Continuous variables are presented as a mean ± standard deviation (SD), and categorical variables as number and percentage (*n*, %). Normality tests were performed using the Kolmogorov-Smirnov test to separate parametric from non-parametric variables. Categorical variables were analyzed using the Chi-Square test with Fisher's exact test correction when necessary, whereas continuous variables were analyzed using the Independent Samples *t*-test for parametric variables and the Mann-Whitney *U* test for non-parametric variables. All tests were two-tailed and significance was taken at $P < 0.05$.

RESULTS

Table 1 shows the baseline characteristics of the patients and the comparison between those who were subjected to tracheostomy and those who were not. Patients were subjected more frequently to tracheostomy if their cause of admission was sepsis (3.5% *vs* 0.0%, $P = 0.001$) and trauma (17.4% *vs* 7.2%, $P < 0.001$) and less frequently if they were admitted due to surgery (1.7% *vs* 22.9%, $P < 0.001$). As far as the cause of intubation, CNS pathology, trauma, and compromised airway led to tracheostomy more frequently (47.0% *vs* 25.1%, $P < 0.001$), (13.9% *vs* 3.3%, $P < 0.001$) and (11.3% *vs* 2.6%, $P < 0.001$) respectively, while the opposite was true for surgery (0.0% *vs* 45.1%, $P < 0.001$). GCS at admission was significantly lower in patients with tracheostomy (9.6 ± 4.4 *vs* 12.2 ± 4.4, $P < 0.001$), while patients with tracheostomy spent significantly more days in ICU (21.8 ± 10.7 *vs* 4.0 ± 6.3, $P < 0.001$). Finally, after ICU discharge, patients with tracheostomy were transferred less frequently to a ward (65.2% *vs* 76.9%, $P = 0.009$) and more frequently to another hospital (9.6% *vs* 1.7%, $P < 0.001$) or a rehabilitation center (6.1% *vs* 0.6%, $P < 0.001$).

Regarding the timing of the tracheostomy, patients were subjected less frequently to early tracheostomy when the reason for performing it was prolonged mechanical ventilation (14.3% *vs* 30.8%, $P = 0.033$). If the reason for performing ST was a facial trauma, early tracheostomy was chosen significantly more frequently (50.0% *vs* 0.0%, $P = 0.033$), while the opposite was true if the reason for performing ST was a goiter (0.0% *vs* 44.4%, $P = 0.033$). Patients with early tracheostomy spent significantly fewer days on mechanical ventilation (15.3 ± 8.5 *vs* 22.8 ± 9.6, $P < 0.001$) and in ICU in general (18.8 ± 9.1 *vs* 25.4 ± 11.5, $P < 0.001$) (Table 2).

As far as the method of performing the tracheostomy, patients who were subjected to PDT were significantly older (62.6 ± 14.0 *vs* 47.6 ± 15.0 years, $P < 0.001$) compared to those who were subjected to ST; they also had a significantly lower GCS at admission (9.2 ± 4.3 *vs* 11.7 ± 4.2, $P = 0.025$), significantly higher APACHE II score at admission (19.6 ± 6.4 *vs* 15.7 ± 7.0, $P = 0.020$) and significantly higher number of co-morbidities (1.9 ± 1.5 *vs* 1.2 ± 1.0, $P = 0.044$). PDT was more preferable if the cause of admission was CNS pathology (62.5% *vs* 26.3%, $P = 0.004$) and less preferable if the cause of admission was trauma or surgery (13.5% *vs* 36.8%, $P = 0.022$) and (0.0% *vs* 10.5%, $P = 0.026$) respectively. PDT was chosen more frequently if the cause of intubation was CNS pathology (53.1% *vs* 15.8%, $P = 0.003$), while the opposite was true if the cause of intubation was a compromised airway (7.3% *vs* 31.6%, $P = 0.008$). PDT was also more frequent in cases of cardiovascular co-morbidity (54.2% *vs* 21.1%, $P = 0.008$), a low level of consciousness as a reason for tracheostomy (46.9% *vs* 21.1%, $P = 0.044$) and a prolonged duration for stay as a reason for early tracheostomy (67.9% *vs* 0.0%, $P < 0.001$), while it was less frequent in cases of a compromised airway as a reason for tracheostomy or a reason for early tracheostomy (9.4% *vs* 36.8%, $P = 0.005$) and (5.7% *vs* 50.0%, $P = 0.002$) respectively. Finally, patients with PDT were discharged with CPAP less frequently than those with ST (0.0% *vs* 11.8%, $P = 0.030$) (Table 3).

Table 1 Baseline characteristics and comparison between patients who were subjected to tracheostomy and those who were not subjected to tracheostomy, *n* (%)

		Total patients	Patients with tracheostomy	Patients without tracheostomy	*P* value
Gender	Male	375/660 (56.8)	72/115 (62.6)	303/545 (55.6)	0.17
	Female	285/660 (43.2)	43/115 (37.4)	242/545 (44.4)	
Age (yr)		60.2 ± 16.8	60.1 ± 15.2	60.2 ± 17.1	0.97
Cause of admission	CNS	345/660 (52.3)	65/115 (56.5)	280/545 (51.4)	0.32
	CVS	28/660 (4.2)	3/115 (2.6)	25/545 (4.6)	0.45
	RS	56/660 (8.5)	11/115 (9.6)	45/545 (8.3)	0.65
	Sepsis	4/660 (0.6)	4/115 (3.5)	0/545 (0.0)	0.001
	Malignancy	20/660 (3.0)	7/115 (6.1)	13/545 (2.4)	0.06
	Trauma	59/660 (8.9)	20/115 (17.4)	39/545 (7.2)	< 0.001
	Surgery	127/660 (19.2)	2/115 (1.7)	125/545 (22.9)	< 0.001
	Metabolic cause	21/660 (3.2)	3/115 (2.6)	18/545 (3.3)	1.00
Cause of intubation	Not intubated	19/660 (2.9)	0/115 (0.0)	19/545 (3.5)	0.06
	CNS	191/660 (28.9)	54/115 (47.0)	137/545 (25.1)	< 0.001
	CVS	31/660 (4.7)	8/115 (7.0)	23/545 (4.2)	0.21
	RS	42/660 (6.4)	10/115 (8.7)	32/545 (5.9)	0.26
	Trauma	34/660 (5.2)	16/115 (13.9)	18/545 (3.3)	< 0.001
	Metabolic cause	34/660 (5.2)	7/115 (6.1)	27/545 (5.0)	0.62
	Compromised airway	27/660 (4.1)	13/115 (11.3)	14/545 (2.6)	< 0.001
	Sepsis	36/660 (5.5)	7/115 (6.1)	29/545 (5.3)	0.74
	Surgery	246/660 (37.3)	0/115 (0.0)	246/545 (45.1)	< 0.001
GCS (N)		11.8 ± 4.5	9.6 ± 4.4	12.2 ± 4.4	< 0.001
APACHE II (N)		19.2 ± 8.0	19.0 ± 6.7	19.3 ± 8.5	0.65
APDR (%)		35.0 ± 24.5	33.2 ± 20.1	35.7 ± 26.1	0.29
Number of co-morbidities (N)		1.8 ± 1.5	1.75 ± 1.40	1.75 ± 1.49	0.97
Co-morbidity	Cardiovascular	329/660 (49.8)	56/115 (48.7)	273/545 (50.1)	0.79
	Metabolic	273/660 (41.4)	46/115 (40.0)	227/545 (41.7)	0.74
	Respiratory	74/660 (11.2)	13/115 (11.3)	61/545 (11.2)	0.97
	Autoimmune	10/660 (1.5)	3/115 (2.6)	7/545 (1.3)	0.39
	Malignancy	83/660 (12.6)	10/115 (8.7)	73/545 (13.4)	0.17
	Psychiatric	46/660 (7.0)	10/115 (8.7)	36/545 (6.6)	0.42
	Renal	41/660 (6.2)	6/115 (5.2)	35/545 (6.4)	0.63
	Neurological	42/660 (6.4)	9/115 (7.8)	33/545 (6.1)	0.48
	Hematological	19/660 (2.9)	3/115 (2.6)	16/545 (2.9)	1.00
	Urological	26/660 (3.9)	3/115 (2.6)	23/545 (4.2)	0.60
	Infectious	10/660 (1.5)	4/115 (3.5)	6/545 (1.1)	0.08
Days in ICU (N)		7.1 ± 9.9	21.8 ± 10.7	4.0 ± 6.3	< 0.001
Transferred to	Ward	494/660 (74.8)	75/115 (65.2)	419/545 (76.9)	0.009
	Another hospital	20/660 (3.0)	11/115 (9.6)	9/545 (1.7)	< 0.001
	Rehabilitation center	10/660 (1.5)	7/115 (6.1)	3/545 (0.6)	< 0.001

	CCU	6/660 (0.9)	3/115 (2.6)	3/545 (0.6)	0.07
	ICCU	12/660 (1.8)	0/115 (0.0)	12/545 (2.2)	0.24
Survival	Yes	542/660 (82.1)	96/115 (83.5)	446/545 (81.8)	0.68
	No	118/660 (17.9)	19/115 (16.5)	99/545 (18.2)	

N: Number; CNS: Central nervous system; CVS: Cardiovascular system; RS; Respiratory system; GCS; Glasgow coma scale; APACHE: Acute physiology and chronic health evaluation; APDR: APACHE predicted death rate, ICU: Intensive care unit; CCU: Critical care unit; ICCU: Intensive coronary care unit.

As far as survival, it was less frequent if the case of admission was CVS pathology, malignant or metabolic disease (2.4% *vs* 12.7%, $P < 0.001$), (1.9% *vs* 8.5%, $P = 0.001$) and (2.0% *vs* 8.5%, $P = 0.001$) respectively, while it was more frequent if the cause of admission was trauma or surgery (10.3% *vs* 2.5%, $P = 0.007$) and (21.2% *vs* 10.2%, $P = 0.006$) respectively. If the patients were not intubated or were intubated due to surgery, they were more likely to survive (3.5% *vs* 0.0%, $P = 0.033$) and (45.2% *vs* 0.9%, $P < 0.001$) respectively, while they were less likely to survive if they were intubated due to CNS, CVS, metabolic, or septic pathology (26.6% *vs* 39.8%, $P = 0.004$), (2.6% *vs* 14.4%, $P < 0.001$), (4.2% *vs* 9.3%, $P = 0.036$) and (2.0% *vs* 21.2%, $P < 0.001$) respectively. The patients who survived presented with a higher GCS (12.6 ± 4.0 *vs* 7.9 ± 4.8, $P < 0.001$), a lower APACHE II score (16.9 ± 7.0 *vs* 25.1 ± 7.3, $P < 0.001$) and a lower number of co-morbidities (1.7 ± 1.5 *vs* 2.1 ± 1.5, $P = 0.005$). Patients were also less likely to survive if they presented with a cardiovascular or a hematological co-morbidity (48.0% *vs* 58.5%, $P = 0.039$) and (2.0% *vs* 6.8%, $P = 0.011$) respectively, while survivors spent less days in ICU compared to non-survivors (6.7 ± 9.9 *vs* 8.9 ± 9.8, $P = 0.028$) (Table 4).

DISCUSSION

Performing tracheostomy in critical care patients in the ICU is a common procedure in the ICU. An interesting finding of this research is that successful weaning from mechanical ventilation was possible in the majority of the patients, as 88 out of 115 patients who underwent tracheostomy managed to be weaned from the ventilator.

Lim *et al*[13] found that weaning parameters measured before and after tracheostomy in difficult-to-wean patients differed significantly. In particular, after tracheostomy, maximum inspiratory pressure, maximum expiratory pressure, and tidal volume significantly increased, whereas rapid shallow breathing index and airway resistance significantly decreased due to the contrast in length and shape between the endotracheal and tracheostomy of tubes, the biofilm formation in the endotracheal tubes and the improved comfort of the patients after tracheostomy.

With regard to the timing of performing a tracheostomy, early tracheostomy was defined as intervention no more than 8 d after initiation of mechanical ventilation. We defined late as tracheostomy placement after 8 d of intubation[8]. It is interesting that the early conversion from an endotracheal tube to tracheostomy had, as a result, a shorter duration of mechanical ventilation in comparison with patients who underwent late tracheostomy with a statistically significant difference. This finding is consistent with a systematic review and meta-analysis of Griffiths *et al*[14] that show that performing a tracheostomy at an earlier stage than is currently practiced may shorten the duration of artificial ventilation and length of stay in intensive care.

As stated by Dochi *et al*[15], who investigated the effect of the timing of tracheostomy in patients who required prolonged mechanical ventilation using two methods: the early *vs* late tracheostomy, for patients requiring ventilation, performing tracheostomy within ten days of admission was independently associated with shortened duration of mechanical ventilation.

General indications for tracheostomy placement include acute respiratory failure with the expected need for prolonged mechanical ventilation, inability to wean from mechanical ventilation, upper airway obstruction, difficult airway, and copious secretions[16].

In our study, CNS pathology combined with low GCS, surgical trauma with subsequent prolonged mechanical ventilation, and compromised airway, mainly due to cranio-maxillofacial injury, led to tracheostomy more frequently than other causes.

A study by Ahmadinegad and co-workers[17] showed that the GCS of patients with severe head injuries on day five following ICU admission might be used for decision-making regarding the time of tracheostomy. A Tracheostomy should be carried out on day five following ICU admission if the GCS is ≤ 8, but it can be delayed if the GCS on the 5th day is > 9.

In the present study, the actual cause for performing an early tracheostomy was a cranio-maxillofacial injury in polytrauma patients and surgical conditions – malignant or not - of the oral cavity, maxillofacial area, and neck. This finding is consistent with the study of Chandrashekar *et al*[18], who described tracheostomy in ICU as an important and safe procedure if prolonged endotracheal intubation is advised for varying underlying causes.

A PDT is usually selected as a method of choice in critical care patients in a particular study. According to a review by Khaja *et al*[19], PDT is a bedside procedure that is safe to perform, has less procedural time, has low cost, and does not need operating schedule time. Also, complications like bleeding and infection are minimal with a percutaneous tracheostomy.

Our study did not observe a statistically significant difference between the two well-known methods of conducting a tracheostomy, namely, the PDT and the open ST approach. de Kleijn *et al*[20] stated that the rate of short- and long-term complications, including tracheal stenosis, is equal in PDT and ST and that PDT is a safe alternative for ST in selected

Table 2 Baseline characteristics of the patients who were subjected to tracheostomy and comparison between those who were subjected to early tracheostomy and those who were subjected to late tracheostomy, *n* (%)

		Whole of the patients	Patients with early tracheostomy	Patients with late tracheostomy	*P* value
Gender	Male	72/115 (62.6)	40/63 (63.5)	32/52 (61.5)	0.83
	Female	43/115 (37.4)	23/63 (36.5)	20/52 (38.5)	
Age (yr)		60.1 ± 15.2	60.1 ± 14.3	60.2 ± 16.4	0.95
Cause of admission	CNS	65/115 (56.5)	38/63 (60.3)	27/52 (51.9)	0.37
	CVS	3/115 (2.6)	1/63 (1.6)	2/52 (3.9)	0.59
	RS	11/115 (9.6)	8/63 (12.7)	3/52 (5.8)	0.34
	Sepsis	4/115 (3.5)	1/63 (1.6)	3/52 (5.8)	0.33
	Malignancy	7/115 (6.1)	2/63 (3.2)	5/52 (9.6)	0.24
	Trauma	20/115 (17.4)	10/63 (15.9)	10/52 (19.2)	0.64
	Surgery	2/115 (1.7)	2/63 (3.2)	0/52 (0.0)	0.50
	Metabolic	3/115 (2.6)	1/63 (1.6)	2/52 (3.9)	0.59
Cause of intubation	CNS	54/115 (47.0)	32/63 (50.8)	22/52 (42.3)	0.36
	CVS	8/115 (7.0)	5/63 (7.9)	3/52 (5.8)	0.73
	RS	10/115 (8.7)	7/63 (11.1)	3/52 (5.8)	0.51
	Trauma	16/115 (13.9)	8/63 (12.7)	8/52 (15.4)	0.68
	Metabolic	7/115 (6.1)	2/63 (3.2)	5/52 (9.6)	0.24
	Compromised airway	13/115 (11.3)	7/63 (11.1)	6/52 (11.5)	0.94
	Sepsis	7/115 (6.1)	2/63 (3.2)	5/52 (9.6)	0.24
	Surgery	0/115 (0.0)	0/63 (0.0)	0/52 (0.0)	N/A
GCS (N)		9.6 ± 4.4	9.3 ± 4.1	10.1 ± 4.6	0.33
APACHE II (N)		19.0 ± 6.7	18.3 ± 6.4	19.8 ± 7.0	0.25
APDR (%)		33.2 ± 20.1	31.7 ± 18.7	35.0 ± 21.7	0.38
Number of co-morbidities (N)		1.75 ± 1.40	1.75 ± 1.33	1.75 ± 1.49	0.99
Co-morbidity	Cardiovascular	56/115 (48.7)	34/63 (54.0)	22/52 (42.3)	0.21
	Metabolic	46/115 (40.0)	22/63 (34.9)	24/52 (46.2)	0.22
	Respiratory	13/115 (11.3)	7/63 (11.1)	6/52 (11.5)	0.94
	Autoimmune	3/115 (2.6)	2/63 (3.2)	1/52 (1.9)	1.00
	Malignancy	10/115 (8.7)	6/63 (9.5)	4/52 (7.7)	1.00
	Psychiatric	10/115 (8.7)	6/63 (9.5)	4/52 (7.7)	1.00
	Renal	6/115 (5.2)	4/63 (6.4)	2/52 (3.9)	0.69
	Neurological	9/115 (7.8)	7/63 (11.1)	2/52 (3.9)	0.18
	Hematological	3/115 (2.6)	1/63 (1.6)	2/52 (3.9)	0.59
	Urological	3/115 (2.6)	1/63 (1.6)	2/52 (3.9)	0.59
	Infectious	4/115 (3.5)	3/63 (4.8)	1/52 (1.9)	0.63
Reason for tracheostomy	Prolonged mechanical ventilation	25/115 (21.7)	9/63 (14.3)	16/52 (30.8)	0.033
	Compromised Airway	16/115 (13.9)	9/63 (14.3)	7/52 (13.5)	0.90
	Low level of consciousness	49/115 (42.6)	30/63 (47.6)	19/52 (36.5)	0.23

	Myopathy	25/115 (21.7)	15/63 (23.8)	10/52 (19.2)	0.55
Method of tracheostomy	PDT	96/115 (83.5)	53/63 (84.1)	43/52 (82.7)	0.84
	ST	19/115 (16.5)	10/63 (15.9)	9/52 (17.3)	
Reason for ST	Facial trauma	5/19 (26.3)	5/10 (50.0)	0/9 (0.0)	0.033
	Cervical burn	1/19 (5.3)	0/10 (0.0)	1/9 (11.1)	0.47
	Difficult airway	6/19 (31.6)	3/10 (30.0)	3/9 (33.3)	1.00
	Cervical edema	1/19 (5.3)	1/10 (10.0)	0/9 (0.0)	1.00
	Goiter	4/19 (21.1)	0/10 (0.0)	4/9 (44.4)	0.033
	Re-opening	2/19 (10.5)	1/10 (10.0)	1/9 (11.1)	1.00
Complications		0/115 (0.0)	0/63 (0.0)	0/52 (0.0)	n/a
VAP		31/115 (27.0)	17/63 (27.0)	14/52 (26.9)	0.99
Pathogen isolated	None	11/47 (23.4)	7/24 (29.2)	4/23 (17.4)	0.34
	Candida albicans	1/47 (2.1)	1/24 (4.2)	0/23 (0.0)	1.00
	Klebsiella pneumoniae	4/47 (8.5)	2/24 (8.3)	2/23 (8.7)	1.00
	Acinetobacter baumanii	15/47 (31.9)	7/24 (29.2)	8/23 (34.8)	0.68
	Pseudomonas aeruginosa	10/47 (21.3)	4/24 (16.7)	6/23 (26.1)	0.49
	Staphylococcus aureus	1/47 (2.1)	0/24 (0.0)	1/23 (4.4)	0.49
	Proteus mirabilis	2/47 (4.3)	0/24 (0.0)	2/23 (8.7)	0.23
	Escherichia coli	1/47 (2.1)	1/24 (4.2)	0/23 (0.0)	1.00
	Enterococcus faecium	1/47 (2.1)	1/24 (4.2)	0/23 (0.0)	1.00
	Hemophilus influenzae	1/47 (2.1)	1/24 (4.2)	0/23 (0.0)	1.00
Days in ICU (N)		21.8 ± 10.7	18.8 ± 9.1	25.4 ± 11.5	0.001
Transferred to	Ward	75/115 (65.2)	39/63 (61.9)	36/52 (69.2)	0.41
	Another hospital	11/115 (9.6)	8/63 (12.7)	3/52 (5.8)	0.34
	Rehabilitation center	7/115 (6.1)	3/63 (4.8)	4/52 (7.7)	0.70
	CCU	3/115 (2.6)	3/63 (4.8)	0/52 (0.0)	0.25
	ICCU	0/115 (0.0)	0/63 (0.0)	0/52 (0.0)	N/A
Condition at discharge	T-piece	82/115 (71.3)	43/53 (81.1)	39/43 (90.7)	0.19
	Speaking valve	1/115 (0.9)	1/53 (1.9)	0/43 (0.0)	0.45
	Tracheostomy closure	5/115 (4.3)	4/53 (7.6)	1/43 (2.3)	0.38
	CPAP	2/115 (1.7)	2/53 (3.8)	0/43 (0.0)	0.50
	BiPAP	1/115 (0.9)	1/53 (1.9)	0/43 (0.0)	1.00
	Pressure ventilator	3/115 (2.6)	2/53 (3.8)	1/43 (2.3)	1.00
	Volume ventilator	2/115 (1.7)	1/53 (1.9)	1/43 (2.3)	1.00
Breathing at discharge	Unassisted	88/115 (76.5)	47/53 (88.7)	41/43 (95.3)	0.29
	Assisted	8/115 (7.0)	6/53 (11.3)	2/43 (4.7)	
Weaning from mechanical ventilation		87/115 (75.7)	48/60 (80.0)	39/50 (78.0)	0.80
Days on mechanical ventilation (N)		18.6 ± 9.7	15.3 ± 8.5	22.8 ± 9.6	< 0.001
Survival	Yes	96/115 (83.5)	53/63 (84.1)	43/52 (82.7)	0.84
	No	19/115 (16.5)	10/63 (15.9)	9/52 (17.3)	

N: Number; CNS: Central nervous system; CVS: Cardiovascular system; RS: Respiratory system; GCS: Glasgow coma scale; APACHE: Acute physiology and chronic health evaluation; APDR: APACHE predicted death rate; VAP: Ventilator associated pneumonia; ICU: Intensive care unit; CCU: Critical care unit; ICCU: Intensive coronary care unit; CPAP: Continuous positive airway pressure; BiPAP: Bi-level positive airway pressure; PDT: Percutaneous

dilatation tracheostomy; ST: Surgical tracheostomy.

Table 3 Comparison between the patients who were subjected to percutaneous tracheostomy and those who were subjected to surgical tracheostomy, *n* (%)

		Patients with PDT	Patients with ST	*P* value
Gender	Male	59/96 (61.5)	13/19 (68.4)	0.57
	Female	37/96 (38.5)	6/19 (31.6)	
Age (years)		62.6 ± 14.0	47.6 ± 15.0	< 0.001
Cause of admission	CNS	60/96 (62.5)	5/19 (26.3)	0.004
	CVS	3/96 (3.1)	0/19 (0.0)	1.00
	RS	9/96 (9.4)	2/19 (10.5)	1.00
	Sepsis	3/96 (3.1)	1/19 (5.3)	0.52
	Malignancy	6/96 (6.3)	1/19 (5.3)	1.00
	Trauma	13/96 (13.5)	7/19 (36.8)	0.022
	Surgery	0/96 (0.0)	2/19 (10.5)	0.026
	Metabolic	2/96 (2.1)	1/19 (5.3)	0.42
Cause of intubation	CNS	51/96 (53.1)	3/19 (15.8)	0.003
	CVS	7/96 (7.3)	1/19 (5.3)	1.00
	RS	8/96 (8.3)	2/19 (10.5)	0.67
	Trauma	12/96 (12.5)	4/19 (21.1)	0.30
	Metabolic	6/96 (6.3)	1/19 (5.3)	1.00
	Compromised airway	7/96 (7.3)	6/19 (31.6)	0.008
	Sepsis	5/96 (5.2)	2/19 (10.5)	0.33
	Surgery	0/96 (0.0)	0/19 (0.0)	N/A
GCS (N)		9.2 ± 4.3	11.7 ± 4.2	0.025
APACHE II (N)		19.6 ± 6.4	15.7 ± 7.0	0.020
APDR (%)		35.1 ± 20.3	23.6 ± 16.0	0.022
Number of co-morbidities (N)		1.9 ± 1.5	1.2 ± 1.0	0.044
Co-morbidity	Cardiovascular	52/96 (54.2)	4/19 (21.1)	0.008
	Metabolic	37/96 (38.5)	9/19 (47.4)	0.47
	Respiratory	10/96 (10.4)	3/19 (15.8)	0.45
	Autoimmune	3/96 (3.1)	0/19 (0.0)	1.00
	Malignancy	10/96 (10.4)	0/19 (0.0)	0.21
	Psychiatric	9/96 (9.4)	1/19 (5.3)	1.00
	Renal	6/96 (6.3)	0/19 (0.0)	0.59
	Neurological	8/96 (8.3)	1/19 (5.3)	1.00
	Hematological	3/96 (3.1)	0/19 (0.0)	1.00
	Urological	3/96 (3.1)	0/19 (0.0)	1.00
	Infectious	4/96 (4.2)	0/19 (0.0)	1.00
Reason for tracheostomy	Prolonged mechanical ventilation	22/96 (22.9)	3/19 (15.8)	0.76
	Compromised airway	9/96 (9.4)	7/19 (36.8)	0.005
	Low level of	45/96 (46.9)	4/19 (21.1)	0.044

	consciousness			
	Myopathy	20/96 (20.8)	5/19 (26.3)	0.56
Timing of tracheostomy	Early	53/96 (55.2)	10/19 (52.6)	1.00
	Late	43/96 (44.8)	9/19 (47.4)	
Reason for early tracheostomy	Prolonged duration of stay	36/53 (67.9)	0/10 (0.0)	< 0.001
	Compromised airway	3/53 (5.7)	5/10 (50.0)	0.002
	Trauma	10/53 (18.9)	3/10 (30.0)	0.42
	Neuromuscular disease	4/53 (7.6)	2/10 (20.0)	0.24
Complications		0/96 (0.0)	0/19 (0.0)	N/A
VAP		25/96 (26.0)	6/19 (31.6)	0.62
Pathogen isolated	None	9/39 (23.1)	2/8 (25.0)	1.00
	Candida albicans	1/39 (2.6)	0/8 (0.0)	1.00
	Klebsiella pneumoniae	3/39 (7.7)	1/8 (12.5)	0.54
	Acinetobacter baumanii	13/39 (33.3)	2/8 (25.0)	1.00
	Pseudomonas aeruginosa	8/39 (20.5)	2/8 (25.0)	1.00
	Staphylococcus aureus	1/39 (2.6)	0/8 (0.0)	1.00
	Proteus mirabilis	1/39 (2.6)	1/8 (12.5)	0.32
	Escherichia coli	1/39 (2.6)	0/8 (0.0)	1.00
	Enterococcus faecium	1/39 (2.6)	0/8 (0.0)	1.00
	Hemophilus influenzae	1/39 (2.6)	0/8 (0.0)	1.00
Days in ICU (N)		22.0 ± 10.9	20.7 ± 9.8	0.64
Transferred to	Ward	65/96 (67.7)	10/19 (52.6)	0.29
	Another hospital	8/96 (8.3)	3/19 (15.8)	0.39
	Rehabilitation center	5/96 (5.2)	2/19 (10.5)	0.33
	CCU	1/96 (1.0)	2/19 (10.5)	0.07
	ICCU	0/96 (0.0)	0/19 (0.0)	N/A
Condition at discharge	T-piece	68/79 (86.1)	14/17 (82.4)	0.71
	Speaking valve	0/79 (0.0)	1/17 (5.9)	0.18
	Tracheostomy closure	5/79 (6.3)	0/17 (0.0)	0.58
	CPAP	0/79 (0.0)	2/17 (11.8)	0.030
	BiPAP	1/79 (1.3)	0/17 (0.0)	1.00
	Pressure ventilator	3/79 (3.8)	0/17 (0.0)	1.00
	Volume ventilator	2/79 (2.5)	0/17 (0.0)	1.00
Breathing at discharge	Unassisted	73/79 (92.4)	15/17 (88.2)	0.63
	Assisted	6/79 (7.6)	2/17 (11.8)	
Weaning from mechanical ventilation		70/91 (76.9)	17/19 (89.5)	0.35
Days on mechanical ventilation (N)		18.9 ± 9.7	17.6 ± 10.0	0.60
Survival	Yes	79/96 (82.3)	17/19 (89.5)	0.74
	No	17/96 (17.7)	2/19 (10.5)	

N: Number; CNS: Central nervous system; CVS: Cardiovascular system; RS: Respiratory system; GCS: Glasgow coma scale; APACHE: Acute physiology and chronic health evaluation; APDR: APACHE predicted death rate; VAP: Ventilator associated pneumonia; ICU: Intensive care unit; CCU: Critical care unit; ICCU: Intensive coronary care unit; CPAP: Continuous positive airway pressure; BiPAP: Bi-level positive airway pressure; PDT: Percutaneous dilatation tracheostomy; ST: Surgical tracheostomy.

Table 4 Comparison between the patients who survived and those who did not survived, *n* (%)

		Survived	Did not survived	*P* value
Gender	Male	302/542 (55.7)	73/118 (61.9)	0.22
	Female	240/542 (44.3)	45/118 (38.1)	
Age (yr)		59.8 ± 16.7	62.2 ± 17.4	0.16
Cause of admission	CNS	288/542 (53.1)	57/118 (48.3)	0.36
	CVS	13/542 (2.4)	15/118 (12.7)	< 0.001
	RS	45/542 (8.3)	11/118 (9.3)	0.72
	Sepsis	4/542 (0.7)	0/118 (0.0)	1.00
	Malignancy	10/542 (1.9)	10/118 (8.5)	0.001
	Trauma	56/542 (10.3)	3/118 (2.5)	0.007
	Surgery	115/542 (21.2)	12/118 (10.2)	0.006
	Metabolic	11/542 (2.0)	10/118 (8.5)	0.001
Cause of intubation	Not intubated	19/542 (3.5)	0/118 (0.0)	0.033
	CNS	144/542 (26.6)	47/118 (39.8)	0.004
	CVS	14/542 (2.6)	17/118 (14.4)	< 0.001
	RS	33/542 (6.1)	9/118 (7.6)	0.54
	Trauma	30/542 (5.5)	4/118 (3.4)	0.34
	Metabolic	23/542 (4.2)	11/118 (9.3)	0.036
	Compromised airway	23/542 (4.2)	4/118 (3.4)	0.80
	Sepsis	11/542 (2.0)	25/118 (21.2)	< 0.001
	Surgery	245/542 (45.2)	1/118 (0.9)	< 0.001
GCS (N)		12.6 ± 4.0	7.9 ± 4.8	< 0.001
APACHE II (N)		16.9 ± 7.0	25.1 ± 7.3	< 0.001
APDR (%)		27.4 ± 20.6	54.4 ± 23.0	< 0.001
Number of co-morbidities (N)		1.7 ± 1.5	2.1 ± 1.5	0.005
Co-morbidity	Cardiovascular	260/542 (48.0)	69/118 (58.5)	0.039
	Metabolic	217/542 (40.0)	56/118 (47.5)	0.14
	Respiratory	56/542 (10.3)	18/118 (15.3)	0.13
	Autoimmune	8/542 (1.5)	2/118 (1.7)	0.70
	Malignancy	68/542 (12.6)	15/118 (12.7)	0.96
	Psychiatric	36/542 (6.6)	10/118 (8.5)	0.48
	Renal	31/542 (5.7)	10/118 (8.5)	0.26
	Neurological	34/542 (6.3)	8/118 (6.8)	0.84
	Hematological	11/542 (2.0)	8/118 (6.8)	0.011
	Urological	23/542 (4.2)	3/118 (2.5)	0.60
	Infectious	9/542 (1.7)	1/118 (0.9)	1.00
Tracheostomy	No	446/542 (82.3)	99/118 (83.9)	0.79
	Yes	96/542 (17.7)	19/118 (16.1)	
Reason for tracheostomy	Prolonged mechanical ventilation	22/96 (22.9)	3/19 (15.8)	0.76
	Compromised airway	13/96 (13.5)	3/19 (15.8)	0.73
	Low level of consciousness	41/96 (42.7)	8/19 (42.1)	0.96

	Myopathy	20/96 (20.8)	5/19 (26.3)	0.56
Timing of tracheostomy	Early	53/96 (55.2)	10/19 (52.6)	0.84
	Late	43/96 (44.8)	9/19 (47.4)	
Reason for early tracheostomy	Prolonged duration of stay	31/53 (58.5)	5/10 (50.0)	0.73
	Compromised airway	7/53 (13.2)	1/10 (10.0)	0.78
	Trauma	10/53 (18.9)	3/10 (30.0)	0.42
	Neuromuscular disease	5/53 (9.4)	1/10 (10.0)	1.00
Method of tracheostomy	PDT	79/96 (82.3)	17/19 (89.5)	0.74
	ST	17/96 (17.7)	2/19 (10.5)	
Reason for ST	Facial trauma	4/17 (23.5)	1/2 (50.0)	0.47
	Cervical burn	0/17 (0.0)	1/2 (50.0)	0.11
	Difficult airway	6/17 (35.3)	0/2 (0.0)	1.00
	Cervical edema	1/17 (5.9)	0/2 (0.0)	1.00
	Goiter	4/17 (23.5)	0/2 (0.0)	1.00
	Re-opening	2/17 (11.8)	0/2 (0.0)	1.00
Complications [N/T, (%)]		0/96 (0.0)	0/19 (0.0)	n/a
VAP		25/96 (26.0)	6/19 (31.6)	0.62
Pathogen isolated	None	8/40 (20.0)	3/7 (42.9)	0.33
	Candida albicans	1/40 (2.5)	0/7 (0.0)	1.00
	Klebsiella pneumoniae	4/40 (10.0)	0/7 (0.0)	1.00
	Acinetobacter baumanii	12/40 (30.0)	3/7 (42.9)	0.66
	Pseudomonas aeruginosa	9/40 (22.5)	1/7 (14.3)	1.00
	Staphylococcus aureus	1/40 (2.5)	0/7 (0.0)	1.00
	Proteus mirabilis	2/40 (5.0)	0/7 (0.0)	1.00
	Escherichia coli	1/40 (2.5)	0/7 (0.0)	1.00
	Enterococcus faecium	1/40 (2.5)	0/7 (0.0)	1.00
	Hemophilus influenzae	1/40 (2.5)	0/7 (0.0)	1.00
Days in ICU (N)		6.7 ± 9.9	8.9 ± 9.8	0.028

N: Number; CNS: Central nervous system; CVS: Cardiovascular system; RS: Respiratory system; GCS: Glasgow coma scale; APACHE: Acute physiology and chronic health evaluation; APDR: APACHE predicted death rate; VAP: Ventilator associated pneumonia; ICU: Intensive care unit; PDT: Percutaneous tracheostomy; ST: Surgical tracheostomy.

patients.

It is worth mentioning that critical care patients with tracheostomy at a younger age are weaned more easily from mechanical ventilation than older patients due to fewer or no co-morbidities, good physical condition, and a better response to the treatment administered. Apart from this, APDR (Adjusted Predicted Death Rate) in our study was lower in the patients with tracheostomy weaned of artificial ventilation, being a prognostic factor of outcome in this group of patients. In addition, the patients who survived presented with a higher GCS, a lower APACHE II score, and a lower number of co-morbidities.

The Length of stay in the ICU in patients with tracheostomy tubes was significantly longer than patients with an endotracheal tube, and most patients weaned were transferred from ICU either to a ward or to a rehabilitation center. Concerning the outcome of patients, a higher proportion of patients who were subjected to tracheostomy survived during their treatment in the ICU, unlike patients with no tracheostomy, who showed a higher mortality rate.

In a retrospective study of Combes *et al*[21], tracheostomy performed in the ICU for long-term mechanically ventilated patients was associated with lower ICU and in-hospital mortality rates even after carefully controlling for ICU admission and day-3 clinical and physiologic differences between tracheostomized and non-tracheostomized patients.

Prolonged mechanical ventilation, longer ICU length of stay, and higher mortality were observed in patients who developed VAP, even in patients with a tracheostomy cannula. Although this finding did not reach statistical significance, it is known that an endotracheal tube is by far the most important risk factor. Host factors such as the severity of the underlying disease, previous surgery, and antibiotic exposure have all been implicated as risk factors for

the development of VAP[22]. The earlier a tracheostomy is performed, the more the risk factors mentioned above can be avoided. Furthermore, when a patient with a tracheostomy tube is weaned from mechanical ventilation and discharged from the ICU, it could be a reasonable strategy for reducing the incidence of VAP.

Consistent with Szakmany *et al*' systematic review and meta-analysis, early tracheostomy does not help to reduce the length of ICU stay or incidence of VAP[6].

A meta-analysis by Griffiths *et al*[14] compared early tracheostomy with either late tracheostomy or prolonged endotracheal intubation. Early tracheostomy (within seven days of invasive mechanical ventilation) did not significantly reduce the risk of VAP or mortality but reduced the number of days on the ventilator and ICU stay.

According to Gadani *et al*[23], the incidence is directly proportional to the duration of mechanical ventilation, and re-intubation is a strong risk factor for the development of VAP. Therefore, the duration of ventilation has to be reduced to get rid of morbidity and mortality associated with mechanical ventilation, which can be achieved by administering a proper weaning protocol and titrating sedation regimens as per the needs of the patients.

Our study has several limitations. First of all, it was performed at a single ICU center with a small sample size and a non-randomized study design (not blinded) because the decision on the timing of the tracheostomy was judged according to the attending physician's opinion and the patient's clinical status. The current study depended on data that were entered into a clinical database and not collected for research, as a result some data would inevitably be missing. Also, certain variables that have the potential to impact the outcome may not have been recorded *etc*[24]. It is often difficult to identify appropriate study and control groups in retrospective studies[25]. Another limitation was the difficulty of accessibility to patients' medical records. Finally, the study did not evaluate long-term outcomes, such as after ICU and hospital discharge and post-decannulation.

CONCLUSION

In the present study, the early tracheostomy cannula aided in the successful weaning of the critical care patient from mechanical ventilation and the subsequent reduction of ICU length of stay. Also, the appearance or absence of VAP seems to affect the time of the patient's stay in the ICU and the outcome, although it was not associated with a lower mortality rate. The PDT is the most common technique in this study compared with the ST method. In terms of survival, it appears to be more affected by factors such as the patient's age, the cause of admission, the cause of intubation, the comorbidities and GCS scale values, the Apache II score, the predicted mortality rate APDR and less than the tracheostomy itself. It seems that early and percutaneous dilatation tracheostomy is more preferable in ICU patients, compared to late and surgical one, however, more studies are needed to indicate which patients require prolonged ventilation support and investigate the clinical benefits of tracheostomy.

FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Minimum 10-year follow-up outcomes of arthroscopic Bankart's repair with metallic anchors: Reliable results with low redislocation rates

Prateek Kumar Gupta, Vishesh Khanna, Nikunj Agrawal, Pratyaksh Gupta

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Prateek Kumar Gupta, Department of Sports Medicine, Sir Ganga Ram Hospital, New Delhi 110060, India

Vishesh Khanna, Department of Trauma and Orthopdaedics, Wirral University Teaching Hospital NHS Foundation Trust, Wirral CH49 5PE, United Kingdom

Nikunj Agrawal, Sports Medicine, Sir Ganga Ram Hospital, Sir Ganga Ram Hospital Marg, Rajinder Nagar, New Delhi 110060, India

Pratyaksh Gupta, Department of Orthopaedics, Sir Ganga Ram Hospital, New Delhi 110060, India

Corresponding author: Vishesh Khanna, DNB, MBBS, MCh, Doctor, Department of Trauma and Orthopdaedics, Wirral University Teaching Hospital NHS Foundation Trust, Arrowe Park Hospital, Arrowe Park Road, Birkenhead, Wirral CH49 5PE, Wirral CH49 5PE, United Kingdom. visheshkhanna85@gmail.com

Abstract

BACKGROUND

With stiff competition from alternative albeit more expensive counterparts, it has become important to establish the applicability of metallic anchors for shoulder instability in the modern era. This can be accomplished, in part, by analysing long-term outcomes.

AIM

To analyse minimum 10-year outcomes from 30 patients following arthroscopic anterior stabilisation using metallic anchors.

METHODS

Prospectively collected data from arthroscopic Bankart repairs performed using metal anchors during 2007P-2010 were retrospectively analysed in this single-surgeon study. Comprehensive data collection included historical and clinical findings, dislocation details, operative specifics, and follow-up radiological and clinical findings including shoulder scores. The primary outcomes were patient-reported scores (Constant, American Shoulder and Elbow Surgeons [ASES], and Rowe scores) and pain and instability on a visual analogue scale (VAS).

RESULTS
A 3% recurrence rate of dislocation was noted at the final follow-up. Total constant scores at 10 years postoperatively measured between 76 and 100 (mean 89) were significantly better than preoperative scores (mean 62.7). Congruous improvements were also noted in the Rowe and ASES scores and VAS at the 10-year review.

CONCLUSION
Reliable long-term outcomes with metallic anchors in surgery for shoulder instability can be expected. Our results provide additional evidence of their continued, cost-effective presence in the modern scenario.

Key Words: Long-term outcomes; Arthroscopic Bankart repair; Metallic anchors; Low failure rates

Core Tip: This paper describes reliable long-term outcomes with metallic anchors in arthroscopic shoulder stabilisation procedures. In an era where newer bioabsorbable alternatives are increasingly become more prevalent in shoulder surgery, it is important not to undermine the established role of metallic anchors. The present study contributes to the literature with evidence of successful long-term outcomes of at least 10 years in managing shoulder instability with metallic suture anchors.

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INTRODUCTION

In 1923, Bankart described a lesion named after him in anteriorly dislocated shoulders wherein the capsule was said to have detached from the fibrocartilage. The technique described by him was based on 4 patients, and the defect was repaired by interrupted silkworm gut sutures[1]. These have evolved from open procedures to arthroscopic techniques with remarkable and ever-improving success rates[2,3]. The popularity of arthroscopic repair is demonstrated by a 90% preference in shoulder surgeons, and this has been on the rise[4]. This comes despite a prevailing heterogeneity in the long-term outcomes with rates of recurrent instability ranging from 3% to 41%[5]. Perhaps a learning curve is responsible, among other factors, for these figures as recurrent dislocation has improved from 30% in 2000-2005[6] to 7.6% in 2004-2008[2]. A paucity of data also exist on the patient-reported clinical outcomes and scores after surgery and their correlation with redislocation rates[7].

Among arthroscopic stabilisation techniques, the use of metallic *vs* bioabsorbable anchors has also been an area of controversy. With prospective randomised studies suggesting no difference in 2-year outcomes, the case for continued employability of the more cost-efficient metallic anchors stands strong[8]. Evidence on survival and outcomes of shoulder stabilisation with metallic anchors for recurrent shoulder dislocation remains sparse. Although bioabsorbable screws have emerged as popular alternatives to avoiding drawbacks with their metallic counterparts, they have not quite phased out the latter.

The present study was designed to evaluate the long-term results of arthroscopic Bankart repair with metallic anchors in shoulder stabilisation for anterior dislocation. We hypothesised that satisfactory outcomes would be seen in the majority of patients undergoing this procedure using these implants.

MATERIALS AND METHODS

Study design

Prospectively collected data of 33 consecutive patients who underwent arthroscopic Bankart repair during 2007-2010 were retrospectively analysed. All patients were between 15 years and 45 years of age and had a diagnosis of recurrent (≥ 2 episodes) traumatic anterior shoulder dislocation. Those with atraumatic dislocations, bony Bankart lesions, multidirectional instability, generalised laxity, co-existing cuff tears, and habitual dislocation were excluded. The single-surgeon study was performed at a tertiary care teaching centre.

Clinical data

Findings from historical and clinical assessments were recorded including demographics, socioeconomics, mode of injury, profession, and hand dominance. Details of each dislocation before and after the Bankart repair, including the need for hospital admission, were also included for analysis. Operative details were assessed from anaesthetic charts, and positioning and operative details including any additional procedures recorded. Data collected at follow-up included a

full upper limb examination including range of movement and tests for shoulder stability. The visual analogue scale (VAS) was used for grading patient satisfaction, and shoulder scores employed for data collection were the Constant score, American Shoulder and Elbow Surgeons (ASES) score, and Rowe score[9-11]. Patients reporting symptoms of pain and stability were also recorded on the VAS with scores of 1 representing the worst pain and instability and 10 representing no persistent symptoms. Radiographs were obtained at sequential reviews and included standard anteroposterior, lateral, outlet, and Stryker notch views.

RESULTS

Among the 33 patients meeting the inclusion criteria, 3 were lost to long-term follow-up. The mean patient age was 25 years with a striking preponderance for the male sex and the dominant arm (Tables 1-3).

Less than half of the patients had between two and five dislocations prior to the stabilisation procedure, while the majority had six or more episodes (Table 4). The time taken to receive surgery was more than 1 year since the first dislocation in the majority of patients (60%) (Table 5).

Sports-related injuries were seen in most patients (80%), while the remaining injuries were divided between motor vehicle accidents and miscellaneous injuries (Table 6).

General anaesthesia was routinely employed for all patients in addition to standard lateral positioning with the arm in abduction holders. Posterior portals were primarily used for viewing, while anteroinferior and anterosuperior portals were used as working portals.

In 80% of patients, the lesion observed intraoperatively was a Bankart between the 3 and 5 o'clock position. An associated non-engaging Hill Sachs lesion was seen in 60% of patients. These were deemed to be small and were not surgically addressed. The use of two metallic anchors was deemed satisfactory intraoperatively in the majority (86.7%) of patients. The others required three anchors. Capsular plication was necessitated in 3 cases.

At a mean of 10 years postoperatively, 3% of patients had a recurrence of dislocation. Among outcomes, total constant scores at 1 year measured between 76 and 100 with a mean score of 85.7, while at 10 years postoperatively these again measured between 76 and 100 with an average of 89 for all 30 patients. These were considerably improved from preoperatively recorded scores (mean 62.7) (Tables 7 and 8).

Similar outcomes were shown by the total Rowe and ASES scores (Tables 9 and 10). When separately evaluated, the ASES score for function displayed a stepped pattern in progressive improvement in the follow-up phase leading up to a mean of 10 years (Table 11).

Overall, most patients were also satisfied at 10 years when asked about symptoms including pain and stability on the VAS scale (Table 12).

At the most recent visits to the clinic (≥ 10 years postoperatively), all patients were negative for clinically apparent drawer, relocation, and load shift tests. Radiographic evaluation at a mean of 10 years did not reveal osteolysis, loosening, failure, or any hardware migration in any of the patients. None of the patients had inadvertent events such as fractures or intraarticular penetration.

Our hypotheses of satisfactory outcomes in the majority of patients undergoing arthroscopic Bankart repair with metal anchors proved accurate.

DISCUSSION

The present study showed reliable long-term results with metallic anchors for anterior shoulder instability. These have faced stiff competition from bioabsorbable screws in arthroscopic shoulder surgery despite no significant differences between the two implants in short- and mid-term outcomes in case-control studies[12]. Metal anchors can, however, potentially result in loosening and prominent hardware in shoulder surgery lest inaccuracies in surgical technique occur [13,14]. Analysing 28 reoperated shoulders with a mean 2.9 anchors per patient, Godinho *et al*[13] reported inadequate anchor positioning in 57% of patients. To obviate complications, a stepwise intraoperative approach starting with the restoration of capsular tension anteroinferiorly with subchondral anchors has been suggested. Also, an appropriate distance of 1-2 mm from the articular margin along a 45° slope has been recommended[15].

Among factors predisposing to early failure, recent research has revealed interesting findings. These factors can be roughly grouped as technical/surgical, patient- and injury-related. Long-term results from 65 arthroscopically stabilised shoulders showed a dislocation rate of 35% in a series by van der Linde *et al*[16]. The authors reported the use of fewer than three anchors and the presence of Hill Sachs lesions as being predictive of redislocation[16]. In a more detailed review, Ho *et al*[17] categorically described patient-related factors responsible for failure as younger male patients with a higher number of preoperative dislocations. Technique-associated errors with recurrences have included superiorised and medialised glenoid anchors, ≤ 2 in number with a poor suturing configuration. Among the missed injuries, Hill Sachs, anterior glenoid defects, humeral avulsion of glenohumeral ligament lesions, and capsular laxity were common causes of failure of stabilisation procedures. Literature suggesting the occurrence of large engaging Hill Sachs lesions has fortunately shown a lower overall incidence of 7% among anterior dislocators[18]. Typically, non-engaging Hill Sachs have been managed non-surgically with good effects and minimal impact on outcomes[19,20]. In the present series, a Hill Sachs lesion was seen in 60% of patients, all of which were non-engaging. The milder severity of these lesions could be one of the reasons for the very low (3%) postoperative recurrence rate of instability in our study at the long-term 10-year follow-ups.

Table 1 Age distribution

Age group in yr	Number of patients	Percentage
< 20	6	20
21-25	10	33.3
26-30	6	20
> 30	8	26.7

Table 2 Sex distribution

Sex	Number of patients	Percentage
Male	28	93.3
Female	2	6.7

Table 3 Hand dominance

Side	Number of patients	Percentage
Dominant	22	73.3
Non-dominant	8	26.7

Table 4 Number of dislocations prior to surgery

Dislocation number	Number of patients	Percentage
< 2	-	-
2-5	14	46.7
6-10	10	33.3
> 10	6	20

Table 5 Time between index dislocation and surgery

Time interval in mo	Number of patients	Percentage
< 2	-	-
2-6	-	-
6-12	12	40
> 12	18	60

Table 6 Mode of injury

Injury mode	Number of patients	Percentage
Road traffic accidents	4	13.3
High energy sports	24	80
Others, miscellaneous	2	6.7

Table 7 Mean shoulder scores

Score	Preoperatively	1-yr postoperatively	10-yr postoperatively
Constant	62.7	85.7	89
Rowe	58	92	98.7
ASES	71.3	90.6	92.4

ASES: American Shoulder and Elbow Surgeons.

Table 8 Constant scores

Score	Number of patients		
ADLs	Preoperatively	1-yr postoperatively	10-yr postoperatively
< 5	-	-	-
5-10	-	-	-
11-15	18	-	-
16-20	12	30	30
ROM	Preoperatively	1-yr postoperatively	10-yr postoperatively
< 25	-	-	-
26-30	4	-	-
31-35	16	-	-
36-40	10	30	30
Total	Preoperatively	1-yr postoperatively	10-yr postoperatively
< 25	-	-	-
26-50	6	-	-
51-75	24	-	-
76-100	-	30	30

ADLs: Activities of daily living; ROM: Range of movement.

Table 9 Total Rowe scores

Score	Preoperatively	1-yr postoperatively	10-yr postoperatively
< 25	-	-	-
26-50	-	-	-
51-75	14	-	-
76-100	16	30	30

Data are *n*.

In the present paper, good long-term outcomes were achieved with two anchors in almost 90% of patients. Emerging evidence has helped clarify the long-held contention of needing > two anchors for success after shoulder stabilisation. In a recent paper from Halifax, Witney-Lagen *et al*[21] demonstrated among 114 postoperative patients no significant differences in recurrent instability and Oxford Instability Scores at mean 4-year follow-ups between recipients of 1 (62.3%), 2 (35.1%), and 3 (2.6%) anchors ($P > 0.05$). Our findings are in accordance with these results.

Higher than expected recurrence rates of 19.1% at 33 mo of follow-up have surfaced from Brazil with the use of metal anchors for shoulder instability in 47 patients. Young age (≤ 20 years) was implicated as the only significant correlator for recurrence[22]. These findings are in contrast to highly satisfactory outcomes reported even with massive 270-degree labral tears at 10 years in young patients (mean age 27.1 years)[23]. We observed similar improvements in Rowe and ASES scores and the VAS at 1- and 10-year follow-ups in the present series (Table 13). Recently published Turkish data

Table 10 Total American Shoulder and Elbow Surgeons scores

Score	Preoperatively	1-yr postoperatively	10-yr postoperatively
< 25	-	-	-
26-50	-	-	-
51-75	14	-	-
76-100	16	30	30

Data are *n*.

Table 11 American shoulder and elbow surgeons score-function

Score	Preoperatively	1-yr postoperatively	10-yr postoperatively
< 30	-	-	-
31-35	-	-	-
36-40	20	-	-
41-45	8	18	4
46-50	2	12	26

Data are *n*.

Table 12 Visual analogue scale scores

Score	Preoperatively	1-yr postoperatively	10-yr postoperatively
< 3	-	-	-
4-7	22	-	-
8-10	8	30	30

Data are *n*.

Table 13 Evidence table – comparing outcomes with relevant literature

Ref.	Number of patients	Year of study	Implants	Follow-up	Recurrence of dislocation, %	Comments
Martel *et al*[22], 2016	47	2010-2012	Metal anchors	33 mo (mean)	19.1	Significant correlation of postoperative recurrence with age ≤ 20 at first dislocation at surgery
Berthold *et al*[23], 2021	21	2003-2010	PEEK	10-yr (minimum)	14.3	Good outcomes with of extensive (270 degree) tears
Uluyardımcı *et al* [24], 2021	67	2009-2016	Metal and all-suture	41 mo (mean)	3	Comparable and reliable outcomes with all-suture anchors and metal anchors
Present study, 2022	30	2007-2010	Metal anchors	10-yr (minimum)	3	Reliable long-term outcomes of Bankart repair with metal anchors

from a mean of 41-mo follow-ups of 67 patients also demonstrated significant improvements in patient-reported outcomes (Rowe, Constant score) with low (3%) redislocation rates, in agreement with present study (3%)[24]. Interestingly, the outcomes published by Uluyardımcı *et al*[24] showed no differences between all-suture and metal anchors used in their study group.

In a systematic review comparatively evaluating the outcomes and complications of absorbable and metallic anchors, Papalia included four randomised studies, two prospective cohort studies, and four case series. The results from this large body of evidence could not offer a superiority of one device over another leaving us with the conclusion of choosing

from the two options largely based on cost-effectiveness[25]. A lateral thought process continuing from the above also questions whether the "drift" to bioabsorbable sutures from the economically viable metallic sutures has actually been driven by scientific evidence[26]. Others have also suggested cognizance towards potential benefits vis a vis cost-effectiveness between newer and time-tested implant materials in shoulder surgery[27].

While we have addressed the lacuna in literature on long-term outcomes of shoulder stabilisation with metallic anchors, we acknowledge the limitations as a part of our research work. These include a relatively small sample size which, however, is very comparable to published literature on long-term 10-year outcomes in shoulder surgery. Also, the non-comparative nature of the paper could not directly draw comparisons between bioabsorbable and metallic anchors which could be addressed in another study design. Despite these limitations, the present research is one of the few if not the first to determine the long-term trustworthiness of repairs with metal anchors for Bankart repairs.

CONCLUSION

The purpose of this research was to illustrate outcomes and results at 10 years following anterior shoulder stabilisation with arthroscopic repair of Bankart lesions with metallic suture anchors. With satisfactory long-term outcomes, we conclude that clinically reliable results can be expected from the surgery provided there is adherence to a consistent technique and routine.

ARTICLE HIGHLIGHTS

Research background

Arthroscopic shoulder surgery is considered the gold standard for anterior and posterior shoulder stability. Among several options of repairing the avulsed labrum, metallic and bio-absorbable anchors are chief competitors. While the latter are considered relatively newer concepts, metallic anchors have stood the test of time. Notwithstanding this, there is a tendency to undermine the role of metallic anchors in the current scenario. This, in part, can be due to the lack of long-term outcomes following stabilisation surgery.

Research motivation

There is no clear evidence of the inferiority of long-term outcomes of metallic anchors vis-a-vis bioabsorbable anchors in shoulder surgery. This gap in literature was the driving force behind the present paper attempting to highlight long-term outcomes of shoulder stabilisation surgeries performed arthroscopically with metallic anchors.

Research objectives

We reported minimum 10-year outcomes off arthroscopic Bankart repair with metal anchors among 30 patients.

Research methods

A thorough evaluation of minimum 10-year results comprising clinical findings, patient-reported scores and radiological reviews was performed in this single-surgeon study.

Research results

Excellent overall outcomes were reported in most patients with only a 3% re-dislocation rate. All of these surgeries were performed using metallic anchors for shoulder stabilisation.

Research conclusions

The findings of this paper provide additional evidence of the role of metallic anchors and their ability to provide reliable outcomes in the long run.

Research perspectives

Further research with even longer follow-up periods, and perhaps a comparative analysis with bio-absorbable counterparts, may be useful for determining the cost-effectiveness of implants in an increasingly cost-conscious global health economy.

FOOTNOTES

Author contributions: Gupta P, Khanna V, Agrawal N, and Gupta P contributed equally to this work; Gupta P, Khanna V, Agrawal N, and Gupta P designed the research; Gupta P and Agrawal N performed the research; Khanna V and Gupta P performed the analyses and wrote the manuscript; All authors have read and approved the final manuscript.

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Country/Territory of origin: United Kingdom

ORCID number: Vishesh Khanna 0000-0002-3800-6956.

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ORIGINAL ARTICLE

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Discovering hidden patterns: Association rules for cardiovascular diseases in type 2 diabetes mellitus

Pradeep Kumar Dabla, Kamal Upreti, Dharmsheel Shrivastav, Vimal Mehta, Divakar Singh

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Pradeep Kumar Dabla, Dharmsheel Shrivastav, Department of Biochemistry, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, Delhi 110002, India

Kamal Upreti, Department of Computer Science, CHRIST, Ghaziabad 201003, India

Vimal Mehta, Department of Cardiology, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, Delhi 110002, India

Divakar Singh, Barkatullah University Institute of Technology, Barkatullah University, Bhopal 462026, India

Corresponding author: Pradeep Kumar Dabla, MD, Professor, Department of Biochemistry, Govind Ballabh Pant Institute of Postgraduate Medical Education and Research, Jawaharlal Nehru Marg, New Delhi, Delhi 110002, India. pradeep_dabla@yahoo.com

Abstract

BACKGROUND

It is increasingly common to find patients affected by a combination of type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD), and studies are able to correlate their relationships with available biological and clinical evidence. The aim of the current study was to apply association rule mining (ARM) to discover whether there are consistent patterns of clinical features relevant to these diseases. ARM leverages clinical and laboratory data to the meaningful patterns for diabetic CAD by harnessing the power help of data-driven algorithms to optimise the decision-making in patient care.

AIM

To reinforce the evidence of the T2DM-CAD interplay and demonstrate the ability of ARM to provide new insights into multivariate pattern discovery.

METHODS

This cross-sectional study was conducted at the Department of Biochemistry in a specialized tertiary care centre in Delhi, involving a total of 300 consented subjects categorized into three groups: CAD with diabetes, CAD without diabetes, and healthy controls, with 100 subjects in each group. The participants were enrolled from the Cardiology IPD & OPD for the sample collection. The study employed ARM technique to extract the meaningful patterns and relationships from the clinical data with its original value.

RESULTS
The clinical dataset comprised 35 attributes from enrolled subjects. The analysis produced rules with a maximum branching factor of 4 and a rule length of 5, necessitating a 1% probability increase for enhancement. Prominent patterns emerged, highlighting strong links between health indicators and diabetes likelihood, particularly elevated HbA1C and random blood sugar levels. The ARM technique identified individuals with a random blood sugar level > 175 and HbA1C > 6.6 are likely in the "CAD-with-diabetes" group, offering valuable insights into health indicators and influencing factors on disease outcomes.

CONCLUSION
The application of this method holds promise for healthcare practitioners to offer valuable insights for enhancing patient treatment targeting specific subtypes of CAD with diabetes. Implying artificial intelligence techniques with medical data, we have shown the potential for personalized healthcare and the development of user-friendly applications aimed at improving cardiovascular health outcomes for this high-risk population to optimise the decision-making in patient care.

Key Words: Coronary artery disease; Type 2 diabetes mellitus; Coronary angiography; Association rule mining; Artificial intelligence

Core Tip: The study is aimed to apply association rule mining (ARM) to discover the correlation between type 2 diabetes mellitus (T2DM) and coronary artery disease (CAD). ARM leverages clinical and laboratory data to the meaningful patterns for diabetic CAD by harnessing the power help of data-driven algorithms. It will assist to optimise the decision-making in patient care by providing new insights into multivariate pattern discovery. Thus, this research facilitates targeted medication for patients to regulate uncontrolled clinical parameters in case of CAD with T2DM.

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INTRODUCTION

Heart disease has become a global threat and considered to be a major cause of mortality and death rate in modern society[1]. The importance of detailed medical assessment cannot be denied; however; in this era of artificial intelligence, if this skillful tool can be given the added advantage of data-driven algorithms and association rule mining (ARM) techniques, it can prove to be helpful to perform this complicated task to estimate the probability of disease based on data and facts provided to the system[2]. In ARM, frequent item sets are built from symptom transactions using a minimum threshold specified by the user, and a confidence level of 0.9 is established by researchers to rank rules. For positively correlated rules, a minimum support and lift of 0.001 and 1, respectively, can be set. Low-confidence and low-support criteria can capture rare or unusual things, while low-support and high-confidence thresholds yield a small number of customer analytics rules[3]. In medical diagnosis, where many symptom combinations are rare, ARM can help identify linked patterns and rules, making symptom analysis more focused[4].

In several previous studies, the authors used ARM techniques to analyze lung cancer data from the SEER program and used hotspots[5,6]. In healthcare, the relationship between diseases is represented by an association rule among symptoms (or diseases), and in medicine it is represented as P→Q, where P and Q are distinct sets of symptoms (or diseases). P is the rule's antecedents; Q is its consequent. Also, known as "if→then", "if" is antecedent, and "then" is consequent. Support, confidence, and lift are used to quantify the effectiveness of generated rules, which are respectively calculated as follows:

Support (P→Q) = Number of patients having P and Q /total number of patients

Confidence (P→Q) = Number of patients having P and Q/number of patients having P

Lift (P→Q) = [(Number of Patients having both P and Q)/(number of patients having P)] (fraction of patients having Q)

Support establishes a rule's frequency (*i.e.*, generality) for a particular data collection. Lift controls the frequency of symptom Q when illness P occurs. Lift determines P and Q's similarity: Independent (= 1), positive (> 1), negative (< 1) [7]. The Hotspot algorithm, as described previously[8,9], follows a straightforward approach. It starts with the input data and proceeds in a depth-first manner using a greedy strategy. At each node, it branches on the attribute that results in the most significant improvement in the target value while respecting the constraints and then repeats the process recursively for each child node. Each node corresponds to a segment of an association, and the target value can be enhanced by

adjusting the target fraction or average target value. To implement the algorithm, the Hotspot program from "Waika to Environment for Knowledge Analysis" (WEKA)[10] is employed.

This program[8,9] provides an overview of disease predictions based on computational intelligence. Available data suggest that neural networks were used for lumen wall evaluation, vessel contour determination, and plaque characterization, optimizing for phase with each vessel segment analysis[11].

Recent advances in data mining techniques have provided unprecedented opportunities to uncover hidden patterns and associations in complex datasets, leading to new insights into the pathogenesis and management of cardiovascular diseases (CVD). Despite the growing interest in this area, there remains a need for more comprehensive studies that explore the intricate relationships between various clinical and genetic factors influencing the development and progression of coronary artery disease (CAD) in type 2 diabetes mellitus (T2DM) patients. By applying ARM to a large dataset of T2DM patients, this study aimed to identify novel associations between clinical and genetic factors that may help in the early detection and management of CAD in this high-risk population. Understanding these hidden patterns could lead to the development of more personalized approaches for the prevention and treatment of CAD in T2DM patients, ultimately improving their overall prognosis and quality of life.

Thus, our work focused on the application of ARM techniques to evaluate the association between angiographic coronary artery stenosis patterns and its severity in diabetic and non-diabetic CAD patients based on their glycemic control. It can prove useful to assist doctors and patients in early detection and intervention of complicated multifactorial disease like CAD.

MATERIALS AND METHODS

This hospital-based cross-sectional study was performed at the Department of Biochemistry in a tertiary care referral cardiac centre in Delhi, India. We analysed the data of 200 consented, age- and sex-matched, angiographically proven patients diagnosed as having CAD with or without diabetes and 100 healthy control individuals.

Data and sample collection

Venous blood samples were collected from consented patients under proper aseptic techniques for the analysis of serum glycated haemoglobin (HbA1c), blood sugar levels, lipids, and other routine parameters. The level of HbA1c was taken as a serum biomarker for diabetes where HbA1c levels more than 6.5% were considered as diabetic and less than 6.5% as non-diabetic[12]. We enrolled patients who had undergone coronary angiography using Judkin's technique[13] to study angiography patterns.

Proposed analytical model and statistical analysis

In the present investigation, the clinical dataset comprised 35 attributes from enrolled patients, with their real/original clinical values serving as input for the proposed model (Table 1). Data extraction and management are illustrated in Figure 1. Utilizing a Hotspot rule-based data mining method, the study successfully resolved the test, leading to the identification of novel and significant symptom patterns in patients with CAD and diabetes.

As far as available literature is concerned, the current study is the main review to extract the most well-known manifestations of cardiac vessel blockade in cases of CAD with diabetes using straightforward yet incredible Hotspot-based mining. A Hotspot learns a bunch of rules that augment or limit an objective variable or worth of interest[6]. The Hotspot algorithm processes real/numeric clinical data, utilizing the actual values of clinical attributes as input to identify associations among attributes. It aims to discover various rules/patterns specifically for patient groups, including CAD with DM, CAD without DM, and healthy control (HC).

In Figure 2, when we used the clinical data of CAD with DM patients and HCs, the rule mining method produced two rules with support scores of 0.1 and 0.33, confidence of 1, and lift of 1.2 for the antecedent (X) Age = 23 and consequent (Y) "Group = CAD-with-DM" in rule 1. All patients had CAD with DM with a confidence level of 1. Similarly, lift 1.2 shows that Sex = F and Age = 23 are connected with "Group = CAD with DM".

RESULTS

Figure 3 displays a dataset with 300 instances, where the "GROUP" attribute signifies "CAD-with-diabetes" at 33.33% support (100 instances). The figure helps doctors recommend medication for controlling clinical parameters, and pharmacists can develop customized medicines. Rules were generated in the analysis with a maximum branching factor of 4 and rule length of 5, requiring a 1% increase in probability for improvement. Notable rules were found, showing strong connections between health indicators and diabetes likelihood, including elevated HbA1C and random blood sugar levels. These rules provide insights into potential risk factors for CAD with diabetes. For instance, if random blood sugar exceeds 175 and HbA1C is above 6.6, the instance is likely classified as "CAD-with-diabetes." Overall, these rules demonstrate high confidence, lift, and conviction, showcasing their strong predictive capability.

Figure 4 shows a dataset with 300 instances, with the target attribute "GROUP" indicating "CAD-without-diabetes" with support value taken as 33.33% (100 instances) of cases. Rules provided outline various conditions, including blood sugar levels, blood pressure, and other biomarkers, each contributing to the prediction of "CAD-without-diabetes" with associated confidence, lift, and conviction measures. For example, one rule specifies that if lymphocytes are less than or

Table 1 Dataset of coronary artery disease with and without diabetes

S. No	Attribute name	Data type	Categorical value
1	Age	Numeric	Minimum = 23 years, maximum = 78 yr
2	Gender	Categorical	Female, male
3	Diet	Categorical	Non-veg, veg
4	Systolic_BP	Numeric	Minimum = 73, maximum = 200
5	Diastolic_BP	Numeric	Minimum = 24, maximum = 120
6	Smoking	Categorical	No, yes
7	Alcoholic	Categorical	No, yes
8	Tobacco	Categorical	No, yes
9	Hypertensive	Categorical	No, yes
10	Disease type	Categorical	Double-vessel, single-vessel, triple-vessel, no-disease
11	Gensini score	Numeric	Minimum = 0, maximum = 136
12	Total cholesterol	Numeric	Minimum = 45, maximum = 381
13	Triglycerides	Numeric	Minimum = 25.2, maximum = 924
14	High density lipoprotein	Numeric	Minimum = 11, maximum = 132
15	Low density lipoprotein	Numeric	Minimum = 15, maximum = 303
16	Very low density lipoprotein	Numeric	Minimum = 5.4, maximum = 184
17	Random blood sugar	Numeric	Minimum = 72, maximum = 683
18	HbA1C (glycated haemoglobin)	Numeric	Minimum = 3.1, maximum = 16
19	Sodium	Numeric	Minimum = 122, maximum = 156
20	Potassium	Numeric	Minimum = 1.8, maximum = 6
21	Haemoglobin	Numeric	Minimum = 1.8, maximum = 17.4
22	WBC	Numeric	Minimum = 1300, maximum = 66000
23	Neutrophils	Numeric	Minimum = 0, maximum = 34.6
24	Lymphocytes	Numeric	Minimum = 0, maximum = 11
25	Monocyte	Numeric	Minimum = 0.026, maximum = 5.26
26	Eosinophils	Numeric	Minimum = 0, maximum = 4.6
27	Platelet count	Numeric	Minimum = 0.67, maximum = 173
28	Left atrium	Numeric	Minimum = 1, maximum = 4.1
29	Aortic root	Numeric	Minimum = 1, maximum = 3.4
30	LVIDd	Numeric	Minimum = 3.9, maximum = 5.7
31	LVIDs	Numeric	Minimum = 1, maximum = 4.7
32	IVSdIVDd	Numeric	Minimum = 0.6, maximum = 1.5
33	PWTd_PWTs	Numeric	Minimum = 0.7, maximum = 1.5
34	Ejection fraction	Numeric	Minimum = 20, maximum = 60
35	Group	Categorical	CAD-with-diabetes, CAD-without-diabetes, healthy control

CAD: Coronary artery disease; LVIDd: Left ventricular internal dimension in diastole; LVIDs: Left ventricular internal dimension in systole; IVSd: Interventricular septal thickness in diastole; IVDd: Left ventricular posterior wall thickness in diastole; PWTs: Posterior wall thickness in systole.

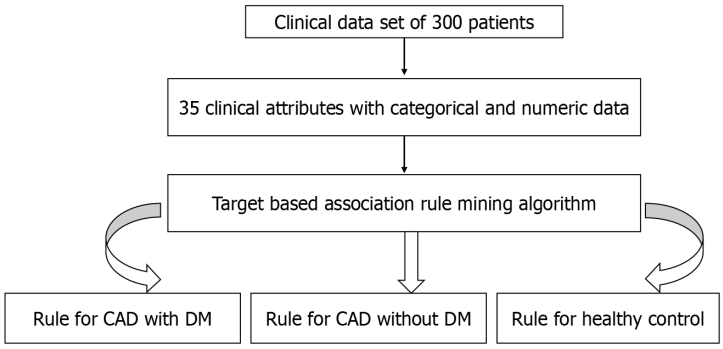

Figure 1 Patient data extraction and management: A flowchart illustrating the process of data extraction and management in the study. CAD: Coronary artery disease; DM: Diabetes mellitus.

Age	Sex	Marital status	Group
23	M	UM	CAD-with-DM
23	M	UM	CAD-with-DM
23	M	UM	CAD-with-DM
23	M	UM	CAD-with-DM
24	M	UM	HC
23	F	UM	CAD-with-DM

Antecedent (X)	Consequents (Y)	Support	Confidence	Lift
Age = 23	CAD-with-DM	0.1	1	1.2
Sex = F	CAD-with-DM	0.1	1	1.2
Age = 23	CAD-with-DM	0.33	1	1.2

Calculating associations using three measures

1. Support: Support (Age = 23) → **CAD-with-DM)** = 5/5 = 1

Here, the support for occurring at age 23 years when **Group as CAD-with-DM** is present is 100%.

2. Confidence

Confidence (Age = 23) → (**CAD-with-DM**) = $\frac{\textbf{Support ((Age = 23), (Group = CAD-with-DM))}}{\textbf{Support (Age = 23)}} = \frac{\textbf{5/5}}{\textbf{5/5}} = 1.00$

Confidence is how likely it is that **Group** = **CAD-with-DM** will occur in patients over the age of 23. In this case, the confidence of occurring in patients over the age of 23 is 100%.

3. Lift

Lift (Age = 23) → $\frac{\text{(CAD-with-DM) = Support ((Age = 23), (CAD-with-DM)) / Support (Age = 23)}}{\text{Support (CAD-with-DM)}} = \frac{(5/5)/(5/5)}{5/6} = 1.2$

Lift assesses the likelihood of when **Group** = **CAD-with-DM** in patients aged 23 and sex = male. Here, the value of lift is 1.2. A lift value greater than 1 implies that it is more likely to occur in patients who had Age = 23 among 6 patients.

Figure 2 Schematic demonstrating calculation of support, confidence, and lift using clinical data for association rule mining. CAD: Coronary artery disease; DM: Diabetes mellitus; M: Male; F: Female; UM: Unmarried.

equal to 2.83, random blood sugar is less than or equal to 167, and HbA1C levels are less than or equal to 6.2, the predicted group is "CAD-without-diabetes." The presented rules demonstrate diverse combinations involving lymphocytes (≤ 5.05), high-density lipoprotein (≤ 96), triglycerides (≤ 559), platelet count (≤ 5.35), diastolic blood pressure (≤ 100), systolic blood pressure (≤ 150), monocyte levels (> 0.189), and sodium (> 125). These rules unveil intricate relationships, providing insights into the factors influencing the presence of CAD without diabetes, along with associated confidence, lift, leverage, and conviction metrics.

Figure 5 shows a dataset that comprises 300 instances, with the target attribute "GROUP" indicating "HC" with support value taken as 33.33%(100 instances). It depicts significant rules, including associations such as [DISEASE_TYPE = no disease, HbA1C ≤ 5.3] and [High density lipoprotein > 58.5, smoking= no], which exhibit robust relationships with the target value HC. The tree incorporates factors like HbA1C levels, lipoprotein concentrations, and lifestyle indicators. Criteria involve conditions such as low HbA1C levels (≤ 4.8), normal blood sugar levels, and specific ranges for lipoproteins. The presented rules highlight connections between various health parameters and the likelihood of disease. These rules offer insights into potential health indicators and contribute to understanding factors influencing disease outcomes within the specified parameters.

The association rules, presented as segment descriptions in Table 2, pertain to clinical feature datasets with support values ranging from 0.33 to 0.99. A confidence level approaching 1 indicates a robust association between clinical attributes and the specified target class (Consequent). Additionally, a lift value exceeding 1 signifies a strong correlation with the clinical attribute[7].

Table 2 Association rules for all groups

Rules	Antecedents ≥ consequents	Support	Confidence	Lift	Segment description
R1	[Random blood sugar > 187, HbA1C > 6.1] ≥ [Group = CAD-with-diabetes]	0.33	1	3	If random blood sugar > 187 and HbA1C > 6.1, then Group = CAD-with-diabetes
R2	[Random blood sugar > 175, High density lipoprotein > 11, HbA1C > 6.1] ≥ [Group = CAD-with-diabetes]	0.66	1	3	If random blood sugar > 175, high density lipoprotein > 11, and HbA1C > 6.1, then Group = CAD-with-diabetes
R3	[Eosinophils ≤ 0.792, sodium ≤ 145, HbA1C > 6.2] ≥ [Group = CAD-with-diabetes]	0.99	0.96	2.88	If eosinophils ≤ 0.792, sodium ≤ 145, and HbA1C > 6.2, then Group = CAD-with-diabetes
R4	[Random blood sugar ≤ 103, Systolic_BP ≤ 130, HbA1C ≤ 6.2] ≥ [Group = CAD-without-diabetes]	0.33	1	3	If random blood sugar ≤ 103, Systolic_BP ≤ 130, and HbA1C ≤ 6.2, then Group = CAD-without-diabetes
R5	[Lymphocytes ≤ 2.83, random blood sugar ≤ 167, HBA1C ≤ 6.4, Systolic_BP ≤ 150] ≥ [Group = CAD-without-diabetes]	0.66	1	3	If lymphocytes ≤ 2.83, random blood sugar ≤ 167, HbA1C ≤ 6.4, and Systolic_BP ≤ 150, then Group = CAD-without-diabetes
R6	[Lymphocytes ≤ 5.05, high density lipoprotein ≤ 96, HBA1C ≤ 6.6] ≥ [Group = cad-without-diabetes]	0.99	0.87	2.61	If lymphocytes ≤ 5.05, high density lipoprotein ≤ 96, and HBA1C ≤ 6.6, then Group = CAD-without-diabetes
R7	[DISEASE_TYPE = No-disease, sodium > 142.5] ≥ [Group = HC]	0.33	1	3	If DISEASE_TYPE = No-disease and sodium > 142.5, then [Group = HC]
R8	[HbA1C ≤ 4.8, low density lipoprotein > 52, DISEASE_TYPE = No-disease] ≥ [Group = HC]	0.66	1	3	If HbA1C ≤ 4.8, low-density lipoprotein > 52, and there is no disease DISEASE_TYPE = No−disease, then [Group = HC]
R9	[DISEASE_TYPE = No-disease, smoking = no, HbA1C ≤ 5.9, tobacco = no] ≥ [Group = HC]	0.99	1	3	If DISEASE_TYPE = No−disease, smoking = no, HbA1C ≤ 5.9, and tobacco = no, then [Group = HC]

Group = CAD-with-diabetes; Group = CAD-without-diabetes; and Group = Healthy Control (HC). HbA1C: Glycated haemoglobin.

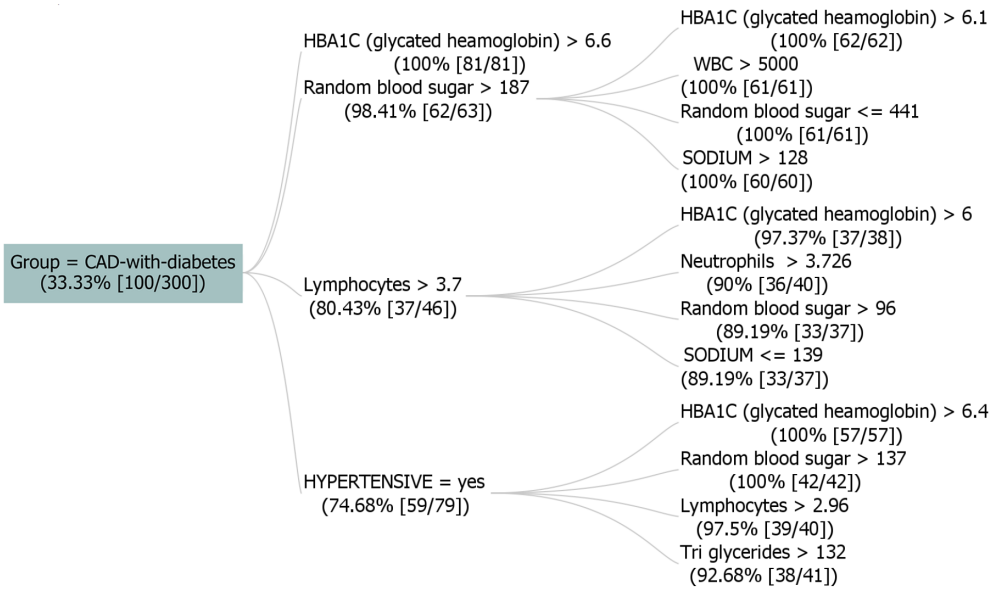

Figure 3 Association rule mining results for patients in the coronary artery disease-with-diabetes group, showing a support of 33%. CAD: Coronary artery disease; HBA1C: Glycated hemoglobin.

DISCUSSION

Oxidative stress and endothelial dysfunction are frequently observed in diabetic patients and may directly contribute to the development of atherosclerosis and CVD[14]. Our study focused on investigating significant differences in diabetic-specific parameters (random blood sugar and serum HbA1c levels) between diabetic and non-diabetic CAD patients. Studies suggested that fasting blood glucose is a major contributor to HbA1c levels in T2DM patients. The underlying multifactorial mechanisms of the disease still pose a challenge[15]. The Hoorn study proposed that T2DM and endothelial dysfunction may interact in a bidirectional manner in the pathogenesis of cardiovascular events[16]. The endothelial dysfunction can be due to various mechanisms, including dyslipidemia, formation of advanced glycated end products, intra-endothelial accumulation of glucose, increased oxidative stress, and low-grade inflammatory responses[17].

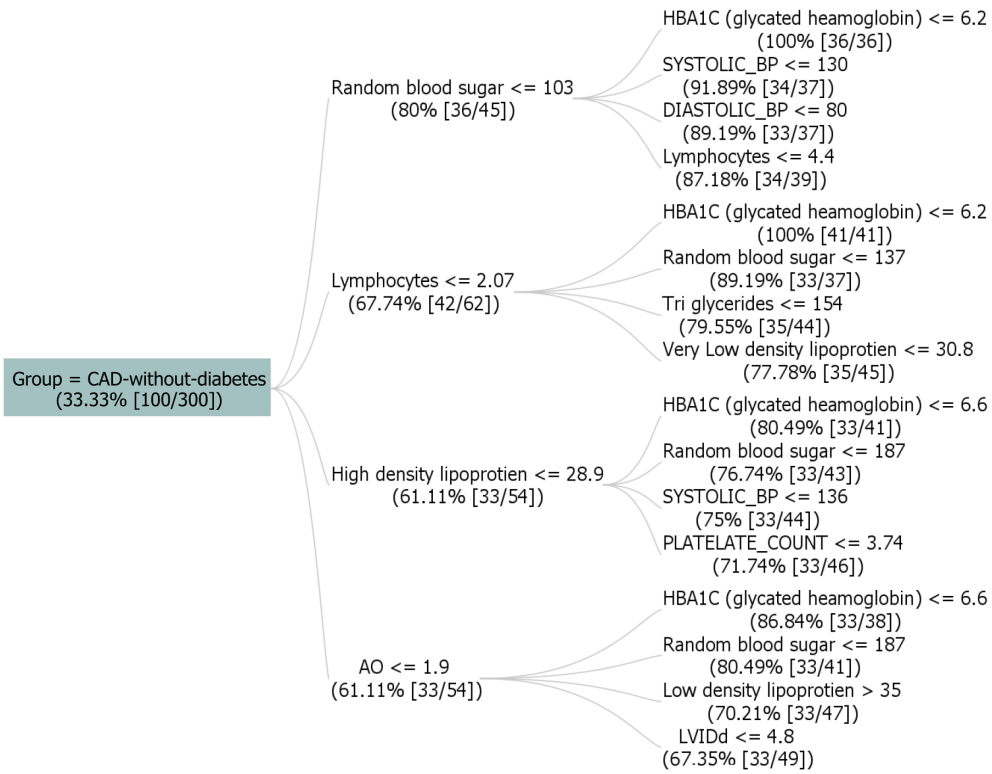

Figure 4 Association rule mining results for patients in the coronary artery disease-without-diabetes group, showing a support of 33%. CAD: Coronary artery disease; HBA1C: Glycated hemoglobin.

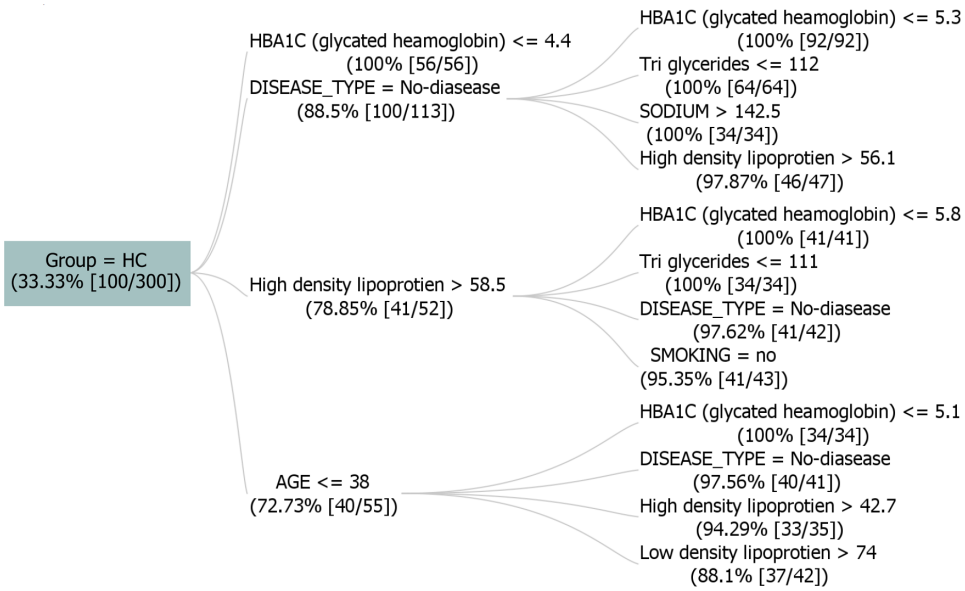

Figure 5 Association rule mining results for subjects in the healthy control group, showing a support of 33%. HC: Healthy control; CAD: Coronary artery disease; HBA1C: Glycated hemoglobin.

Recent advancements in cardiovascular research have shown promising developments. Chen *et al*[18] utilized self-supervised learning to transfer knowledge of cardiovascular progression between cohorts, enhancing the detection of specific cardiovascular events and improving examination strategies and prognosis. Ma *et al*[19] introduced a highly accurate and lightweight deep learning model for phonocardiogram classification, crucial for screening valvular heart disease *via* mobile health applications, demonstrating exceptional accuracy and robustness.

In another study, six machine learning algorithms demonstrated comparable diagnostic accuracy, suggesting stress-only myocardial perfusion imaging supported by machine learning models as a promising alternative in clinical settings [20]. Lee *et al*[21] developed a deep learning model for detecting obstructive CAD on coronary computed tomography angiography, showing high diagnostic accuracy and improved discrimination. Veroneze *et al*[22] applied ARM to

discover consistent patterns of clinical features and differentially expressed genes relevant to T2DM, dyslipidemia, and periodontitis, offering insights into these chronic inflammatory diseases. Reti-CVD, an artificial intelligence (AI) software as a medical device utilizing retinal images for personalized CVD risk assessment, demonstrated a significant association with increased CVD risk, leading to regulatory authorization[23]. Additionally, Singh *et al*[24] validated the ARM method, discovering hidden patterns and associations of early-onset myocardial infarction with hypertension and diabetes mellitus.

Our findings align with previous studies regarding the relationship between T2DM, endothelial dysfunction, and adverse cardiovascular outcomes[25]. Pathak *et al*[26] observed a significantly higher occurrence of triple vessel disease/multivessel disease in patients with a diabetes mellitus duration > 10 years compared to those with a duration < 10 years.

In summary, our study contributes to the growing body of evidence supporting the association between T2DM, endothelial dysfunction, and cardiovascular outcomes. Further research leveraging advanced technologies and methodologies, as demonstrated in recent studies, holds promise for improving cardiovascular risk assessment and management in diabetic patients.

A larger multicenter cohort clinical trial, spanning diverse geographic locations and ethnicities, would be beneficial. Nevertheless, our study lays the groundwork for future large-scale studies that can help healthcare providers establish plasma glucose testing goals based on specific HbA1c values, thereby improving patient care.

CONCLUSION

In conclusion, we demonstrated that ARM is a powerful data analysis technique to identify consistent patterns between the clinical profiles of patients affected by specific pathological panels. In addition, ARM is able to evidence relevant associations among important parameters of HbA1C and random blood sugar levels of the patients. These insights shed light on potential risk factors for cardiovascular disease with diabetes. A standout rule suggests that instances with random blood sugar > 175 and HBA1C > 6.6 are likely in the "CAD with diabetes" group. These findings can contribute to the valuable insights into health indicators and factors influencing disease outcomes. With the aid of this research, doctors can effortlessly prescribe medication to regulate uncontrolled clinical parameters, while pharmacists can innovate customized medicines tailored to address specific attributes with uncontrolled clinical parameters. This research facilitates targeted medication for patients. Thus, it is pertinent to widen the horizon of AI applications, while integrating with CAD research and development in terms of systems development, process modelling, and its optimization.

FOOTNOTES

Author contributions: Dabla PK designed the research; Dabla PK and Shrivastav D performed the research; Mehta V contributed to patient clinical data & sample collection; Upreti K and Shrivastav D contributed analytic tools and analyzed the data; Upreti K, Singh D, and Dabla PK wrote the paper; all authors have accepted responsibility for the entire content of this manuscript, and reviewed and approved its submission.

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Country/Territory of origin: India

ORCID number: Pradeep Kumar Dabla 0000-0003-1409-6771.

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ORIGINAL ARTICLE

Prospective Study

Digestive and breast cancer patients managed during the first wave of COVID-19 pandemic: Short and middle term outcomes

Jacobo Trébol, Ana Carabias-Orgaz, María Carmen Esteban-Velasco, Asunción García-Plaza, Juan Ignacio González-Muñoz, Ana Belén Sánchez-Casado, Felipe Carlos Parreño-Manchado, Marta Eguía-Larrea, José Antonio Alcázar-Montero

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Jacobo Trébol, María Carmen Esteban-Velasco, Asunción García-Plaza, Juan Ignacio González-Muñoz, Ana Belén Sánchez-Casado, Felipe Carlos Parreño-Manchado, Marta Eguía-Larrea, José Antonio Alcázar-Montero, Cirugía General y del Aparato Digestivo, Complejo Asistencial Universitario de Salamanca, Salamanca 37007, Salamanca, Spain

Jacobo Trébol, María Carmen Esteban-Velasco, Juan Ignacio González-Muñoz, Felipe Carlos Parreño-Manchado, Marta Eguía-Larrea, José Antonio Alcázar-Montero, Cirugía, Universidad de Salamanca, Salamanca 37007, Salamanca, Spain

Ana Carabias-Orgaz, Oftalmología, Complejo Asistencial Universitario de Salamanca, Salamanca 37007, Salamanca, Spain

Corresponding author: Jacobo Trébol, MD, PhD, Reader (Associate Professor), Surgeon, Surgical Oncologist, Cirugía General y del Aparato Digestivo, Complejo Asistencial Universitario de Salamanca, Paseo de la Transición Española S/N, Salamanca 37007, Salamanca, Spain. jacobotrebol@gmail.com

Abstract

BACKGROUND

The first wave of coronavirus disease 2019 (COVID-19) pandemic in Spain lasted from middle March to the end of June 2020. Spanish population was subjected to lockdown periods and scheduled surgeries were discontinued or reduced during variable periods. In our centre, we managed patients previously and newly diagnosed with cancer. We established a strategy based on limiting perioperative social contacts, preoperative screening (symptoms and reverse transcription-polymerase chain reaction) and creating separated in-hospital COVID-19-free pathways for non-infected patients. We also adopted some practice modifications (surgery in different facilities, changes in staff and guidelines, using continuously changing personal protective equipment…), that supposed new inconveniences.

AIM

To analyse cancer patients with a decision for surgery managed during the first wave, focalizing on outcomes and pandemic-related modifications.

METHODS

We prospectively included adults with a confirmed diagnosis of colorectal, oesophago-gastric, liver-pancreatic or breast cancer with a decision for surgery, regardless of whether they ultimately underwent surgery. We analysed short-term outcomes [30-d postoperative morbimortality and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection] and outcomes after 3 years (adjuvant therapies, oncological events, death, SARS-CoV-2 infection and vaccination). We also investigated modifications to usual practice.

RESULTS

From 96 included patients, seven didn't receive treatment that period and four never (3 due to COVID-19). Operated patients: 28 colon and 21 rectal cancers; laparoscopy 53.6%/90.0%, mortality 3.57%/0%, major complications 7.04%/25.00%, anastomotic leaks 0%/5.00%, 3-years disease-free survival (DFS) 82.14%/52.4% and overall survival (OS) 78.57%/76.2%. Six liver metastases and six pancreatic cancers: no mortality, one major complication, three grade A/B liver failures, one bile leak; 3-year DFS 0%/33.3% and OS 50.0%/33.3% (liver metastases/pancreatic carcinoma). 5 gastric and 2 oesophageal tumours: mortality 0%/50%, major complications 0%/100%, anastomotic leaks 0%/100%, 3-year DFS and OS 66.67% (gastric carcinoma) and 0% (oesophagus). Twenty breast cancer without deaths/major complications; 3-year OS 100% and DFS 85%. Nobody contracted SARS-CoV-2 postoperatively. COVID-19 pandemic–related changes: 78.2% treated in alternative buildings, 43.8% waited more than 4 weeks, two additional colostomies and fewer laparoscopies.

CONCLUSION

Some patients lost curative-intent surgery due to COVID-19 pandemic. Despite practice modifications and 43.8% delays higher than 4 weeks, surgery was resumed with minimal changes without impacting outcomes. Clean pathways are essential to continue surgery safely.

Key Words: COVID-19; SARS-CoV-2; Colon cancer; Rectal cancer; Breast cancer; Liver cancer; Pancreatic cancer; Gastric cancer; Oesophageal cancer; Surgery

Core Tip: In our department, during coronavirus disease 2019 (COVID-19) first wave, all surgery was discontinued and resumed later for cancer patients. To minimise perioperative severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections, we established a physically separated clean pathway and adopted some practice modifications. We evaluated 96 malignancies; 88 underwent surgery (one SARS-CoV-2 positive): 49 colorectal, 20 breast, 12 liver-pancreatic and 7 oesophago-gastric. Three never received surgery because they contracted COVID-19. 78.2% were treated in alternative buildings, 43.8% waited more than 4 wk, two additional stomas were constructed, and laparoscopy decreased. None contracted perioperative SARS-CoV-2. Clean pathways are essential to continue cancer surgery during pandemics. Despite practice changes, we obtained comparable to standard outcomes.

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INTRODUCTION

At the end of 2019, a novel coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was discovered in China. It causes coronavirus disease 2019 (COVID-19). The virus spread rapidly, and on 11 March 2020, the World Health Organization declared a global pandemic.

In Spain, the health crisis broke out during the first two weeks of March 2020 and officially lasted until July 5, 2023. There were six main waves until March 28, 2022 and, according to official data, it caused about 122000 deaths[1]. The first wave of the COVID-19 pandemic in Spain lasted from the beginning of the outbreak until June 21, 2020. The Spanish Government declared a state of alarm and total confinement of the population on March 14, 2020, with closure of the borders until 26 April. On 2 May, a de-escalation plan was approved according to epidemiological data, with four phases during the following 6–8 wk.

To better understand the first wave in Spain, we highlight two pieces of data: (1) The number of deaths ranged from 27127 (Health Department) to 45684 [Statistics National Institute (INE)][2], considering a total population in March 2020 of 47318050 people[3]. The discrepancy in these numbers could be due to the significant rise in observed deaths (compared with other years). It is suspected that many people died at home without any diagnostic test, and these data were considered by some institutions such as the INE; and (2) Until 10 May 2020, the last day these data were published

separately, there were 40961 infections in healthcare professionals, of whom 4188 (10.5%) required hospital admission, 310 (1.1%) management in intensive care units (ICU) and 52 (0.1%) died[4].

It is also necessary to contextualise the situation concerning patients with digestive and breast cancers and their surgical treatment during the first wave. Especially during March and April 2020, the public health system experienced a massive influx of patients with COVID-19, which overwhelmed hospitals. Healthcare managers had to adopt some measures to preserve and expand wards and ICU beds such as modifying the hospital structure and organisation, opening field hospitals and assigning professionals from specific areas and specialties to the care of these patients. This led to variable cancellation of ordinary activities such as outpatient consultations and scheduled surgeries, to free up staff, resources (*e.g.* ventilators) and space (*e.g.* operating rooms were converted to ICUs). The initial calculations, based on mathematical models, of expected surgery cancellations or delays during the initial 12 wk of the COVID-19 pandemic were 72.3% globally, including 37.7% of cancer-related surgeries (2324070 of 6162311)[5]. So, access to cancer diagnosis and treatment was limited.

During the early period of the COVID-19 pandemic, healthcare workers were considered essential, and infection among them was frequent. Thus, surgeons were advised to take precautions to avoid exposure to COVID-19, including cancelling non-urgent and non-cancer surgeries. Moreover, in the beginning, there were concerns about possible virus aerosolisation with surgical smoke and pneumoperitoneum (based on previous reports with other viruses)[6]. This led some influential scientific societies such as the Association of Surgeons of Great Britain and Ireland to temporarily recommend against laparoscopy, a recommendation that was subsequently corrected. Later, a consensus was reached: It was considered safe increasing the usual preventive measures and implementing others[7] including specific personal protective equipment (PPE), smoke evacuators, aspirate the pneumoperitoneum and test patients before surgery. We modified our protocols to follow these guidelines. Some of these measures (such as PPE) were simplified later as our understanding of SARS-CoV-2 pathophysiology improved. It is also important to highlight that there was a widespread shortage of PPE, including in our setting[8]. These changing recommendations likely affected the care we could give to our patients.

Another issue that concerned surgeons was the possible perioperative morbimortality among patients infected by SARS-CoV-2 undergoing major cancer surgery. The first reports from China with surgical patients were quite worrisome. A major international collaborative scientific effort, the CovidSurg project, was undertaken to increase knowledge of how perioperative infection could affect surgical patients. The preliminary reports and the first publication at the end of May 2020 showed a 30-d mortality rate as high as 23.8%[9], so cancellation or postponement seemed to be a safe option for patients with cancer awaiting surgery. Another issue was to determine whether deferring cancer surgeries is safe and for how long. Clinical judgment argued against long delays, mainly for aggressive tumours such as hepatobiliopancreatic or oesophago-gastric cancer, time-critical diseases for which treatment delays are associated with disease progression and worse outcomes[10,11]. Many oncological associations opposed cancellations or delays[12] and proposed triage guidelines based on expert opinions rather than evidence-based data. Shinde *et al*[13] reviewed the studies addressing the impact of the waiting time to initiate therapy on cancer survival and prognosis and those triage recommendations. Several publications analysed these issues during the first wave of the COVID-19 pandemic, with continuous changes that added some confusion.

In this context, we had to manage patients awaiting their cancer surgery when the COVID-19 pandemic began. In addition, although there was a significant decrease (even cancellation on many days) of diagnostic tests, we had to face the new diagnoses of cancer with the abovementioned impediments.

Our hospital complex is a public university, teaching and fourth-level centre that provides health care to a province of 329245 people (on January 1, 2020). At that time, it comprised two acute care centres very close to each other with approximately 800 beds. Each one had its own critical care beds, postsurgical units, operating rooms and radiology equipment, while other resources (*e.g.* laboratory and blood bank) were shared. The General and Digestive Tract Surgery Department worked in the larger building. At specific moments of the first wave of the COVID-19 pandemic, some operating theatres and hospitalisation beds from a private centre were also employed. During the first wave, our hospital's official data indicate that 1186 proven SARS-CoV-2-positive patients were admitted in conventional wards and 112 in ICUs, with 364 declared in-hospital deaths. The peak number of admitted infected patients was 382 in wards (as of April 3) and 56 in ICUs (as of April 5)[14].

This structure with two well-equipped acute care centres allowed our healthcare managers to allocate the larger building to patients with COVID-19 and the other to patients without COVID-19, with a clear physical barrier between the patient groups. The general and digestive surgery consultants (unlike all of our residents) were not assigned to COVID-19 wards, but attended emergencies not knowing whether the patients were infected and also surgical SARS-CoV-2 positive patients thus using the PPE dictated by the authorities and according to availability. During this period, 8 of the 29 consultants (27.6%) and 5 of the 9 resident doctors (55.6%) in our department contracted COVID-19 and were on sick leave[8]. However, both the anaesthesiologists and the nursing staff (ward and operating room) were assigned according to the needs to SARS-CoV-2-positive or SARS-CoV-2-negative patients.

In this context, we had to implement some modifications to continue treating our patients with cancer: patients not infected by SARS-CoV-2 were managed in a different building, with different operating theatres and wards. Although we tried to ensure that each patient was operated on by the usual subspecialised unit, due to sick leave this was sometimes not feasible for all the surgeries. In addition, we could not work with the usual anaesthesiologists and nurses. We faced another specific limitations: Sometimes the surgical devices and instruments were not what we usually used, and sometimes the recommended PPE had negative effects (heat, fog, weight, pressure, *etc.*) that made surgery difficult, as mentioned in some studies[15,16].

Our department cancelled all elective surgeries for 2 wk to analyse the evolution of COVID-19 hospitalisations. Surgery resumed at the beginning of April but only for patients with cancer who were previously screened for COVID-19 symptoms and with a polymerase chain reaction (PCR) test 48 h before surgery. If the PCR test result was positive, then the surgery was delayed for at least 4–8 wk, and the subsequent PCR test results had to be negative. During the first week of resumption, surgical activity was approximately 10% of the normal level. In the following 2 wk, it was at 10%–20% of the normal level and later stabilised at approximately 33%, when the COVID-19 admissions curve had flattened. By the beginning of June 2020, surgical activity exceeded 50% of the normal activity and some interventions returned to the larger building, in clearly demarcated COVID-19-free pathways. At the end of the first wave, activity returned to normal and in the usual building.

Initially, only surgeries for patients with cancer pathology was scheduled. Later, surgical programs were completed adding some benign cases with clinical priority. In mid-June some sessions with exclusively benign pathology were scheduled. Regarding cancer, we started with the pathology with the lowest complication risk and ICU bed need (colorectal and breast). When the COVID-19 admissions curve had stabilised, we resumed liver-pancreatic and oesophago-gastric surgeries, first treating those patients with the highest probability of long-term survival, with the lowest perioperative risks and with the most symptoms. Our goal was to offer our patients with cancer the same treatment they would have received without a health crisis.

In the present study, we analysed patients with digestive and breast cancer who had a decision for surgical resection managed at our department during the first wave of the COVID-19 pandemic. We focused on the outcomes and the possible impacts of the COVID-19 pandemic.

MATERIALS AND METHODS

Study design

This prospective observational single-centre cohort study included consecutive elective and emergent patients with a confirmed diagnosis of digestive and breast cancer whose pre-COVID-19 pandemic therapeutic decision was surgical management, mostly with a curative intent, regardless of whether they finally had an operation. The study was approved by the local ethical review board (code 2020 04 479) and collected only anonymised data according to Spanish (organic law 3/2018 and law 41/2002) and European Union (EU regulation 2016/679) legislation on data protection and patient autonomy. Due to contagion risks and the policy of minimising personal contacts and physical documentation during the study period, an exception was made regarding obtaining written consent from some patients.

The short-term follow-up data are included in the National Institute for Health Research (NIHR) Global Research Unit on Global Surgery CovidSurg-Cancer, an international research collaborative[17]. The study was conducted according to the guidelines set by the Strengthening The Reporting of Observational Studies in Epidemiology (STROBE) statement for observational studies[18].

Patient inclusion criteria

The study included consecutive adult patients, aged ≥ 18 years, with a diagnosis of digestive (colorectal, oesophago-gastric and hepatobiliary) and breast cancer who were awaiting surgery when the COVID-19 pandemic was declared in Spain (March 14, 2020) and all the patients diagnosed during the first wave in Spain (until 21 June) at our centre. We considered all patients whose pre-pandemic therapeutic decision was surgery with a curative intent, regardless of whether they ultimately underwent surgery.

Outcome measures

The short-term outcomes (for patients who underwent surgery) included the 30-day postoperative COVID-19 infection rate, postoperative mortality and complications. The long-term outcomes included adjuvant treatments received, cancer-related events (relapse and metastases), time to relapse, death, time to death, cause of death, and COVID-19 vaccination and infection and its consequences.

Analysed variables

The *baseline variables* to account for perioperative and SARS-CoV-2 risk included patient demographics (age and sex), comorbidities, American Association of Anesthesiologists (ASA) grade, Eastern Cooperative Oncology Group performance and body mass index.

The cancer baseline details included the location, baseline tumor-node-metastasis (TNM) staging, date of diagnosis and treatment decision by a multidisciplinary team (MDT). Some cancers included more detailed data about histology (*e.g.* breast), options for resection (*e.g.* the National Comprehensive Cancer Network classification for pancreatic cancer) or preoperative treatments [*e.g.* biliary drainage in the pancreas; transarterial chemoembolisation (TACE) or radiofrequency ablation (RFA) in the liver].

The details for patients who underwent surgery included operative characteristics (procedure[s], urgency, approach, extent of resection, margin status, final TNM staging and final surgical intent), additional histological data in patients with breast cancer (oncotype), COVID-19 hospital situation on the day of surgery (according to the CRITCON levels), prior SARS-CoV-2 infection, changes potentially related to the COVID-19 pandemic (*e.g.* in patients with breast cancer whether reconstruction was performed immediately or deferred or in patients with colorectal cancer whether anastomosis was performed, *etc.*). The CRITCON levels were initially designed in London in response to the H1N1 (2009)

influenza pandemic for critical care rationing. In 2020, the Intensive Care Society proposed CRITCON-Pandemic levels as a modification to face the needs and challenges of the COVID-19 pandemic[19,20].

Information collected of the short-term postoperative course: SARS-CoV-2 infection (defined by a laboratory test), mortality, reoperations, general and surgical complications [classified with the Clavien–Dindo (C-D) system[21]] and complications specific to liver and pancreas surgery [postoperative bleeding, bile leak, pancreatic fistula, post-hepatectomy liver failure (PHLF) and cholangitis]. Bile leak and PHLF were defined according to the International Study Group of Liver Surgery criteria[22,23]. Postoperative pancreatic fistula (POPF) was defined according to the International Study Group on Pancreatic Surgery criteria[24]. The postoperative length of stay was also analysed.

For patients who did not undergo an operation during the first wave we assessed whether the indication for surgery persisted and if it was performed, the reason(s) for changes in management, if new re-staging was done and changes attributable to the COVID-19 pandemic.

Information collected regarding the long-term follow-up: Adjuvant treatments; oncological events (local and regional relapses and metastases); time to relapse [disease-free survival (DFS) from surgery or diagnosis in non-operated]; whether the patient was alive or dead at the end of follow-up; reason for death; time to death [overall survival (OS)]; COVID-19 vaccination, doses and dates; and SARS-CoV-2 infection, type and sequelae.

Data collection

Data were collected and managed by clinicians according to a prespecified protocol. The data were uploaded into a secure online Research Electronic Capture Database (REDCap)[25,26] hosted at University of Birmingham (UK) for the short-term follow-up and stored in a secure local Excel database for the long-term follow-up. No data identifying patients were uploaded.

Statistical analysis

SPSS v.28.0.0.1 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis. The categorical data are presented as frequencies and percentages. Continuous data are summarised using the mean and standard deviation (SD) or median and interquartile range depending on their distribution. No analytic tests were performed due to the descriptive nature of the study, the small number of patients in some groups and because different periods were not compared.

RESULTS

This study included 96 patients with cancer managed in our department (54 colorectal, 20 breast, 9 oesophago-gastric, 7 liver and 6 pancreas). When the COVID-19 pandemic was declared, 40 patients were waiting for surgery (18 colorectal, 16 breast, 2 oesophago-gastric, 2 liver and 2 pancreas), so the other 56 were diagnosed during the first wave of the pandemic. Seven patients did not receive the planned treatment. Only one patient with perforated rectal cancer was operated on despite being SARS-CoV-2 positive.

Patients with colorectal cancer

Of the 54 patients, 33 had colon cancer and 21 rectal cancers. Considering the 53 SARS-CoV-2-negative patients, 49 (92.5%) – 29 with colon cancer and 20 with rectal cancer – underwent surgery during the first wave of the pandemic.

The patient demographic data and comorbidities are summarised in Tables 1 and 2. The most frequent comorbidities were hypertension (54.7%), diabetes (23.8%) and moderate to severe chronic kidney disease (13.2%). All patients had at least one comorbidity (mean 3.13, SD 1.34, range 1–6).

The cancer and surgery characteristics (including stoma and anastomosis features) are summarised separately for colon and rectal cancer in Table 3. The surgical procedures, operating surgeon and neoadjuvant therapies are described as follows: Colon cancer procedures (Figure 1A): Right colectomy (12/29, 41.4%), sigmoidectomy with anastomosis (6/29, 20.7%) or stoma (3/29, 10.3%), left colectomy (3/29, 10.3%), extended right colectomy (3/29, 10.3%) and total colectomy with ileorectostomy (1/29, 3.45%). Moreover, two partial cystectomies, one segmental ileal resection, one prosthetic eventroplasty and one cholecystectomy were associated in four patients. Fifteen of 25 anastomoses (60.0%) were handsewn and 10 (40%) were stapled. Non-colorectal consultants and colorectal trainees performed three (10.7%) and two (7.1%) of the procedures, respectively, with fewer trainees performing surgeries compared with the pre-COVID-19 pandemic period. The rectal cancer procedures (Figure 1B) included abdominoperineal resection (9/20, 45.0%) and anterior resection with anastomosis (8/20, 40.0%) or ostomy (3/20, 15.0%). In addition, one hepatic metastasis procedure was performed, and one patient required an ileal segment resection. All anastomoses were stapled. Colorectal consultants (no trainees, a change in usual practice) performed all surgeries. Twelve of the 20 patients (60.0%) received neoadjuvant therapies: long-course chemoradiation (8/12, 67.7%), short-course radiotherapy (2/12, 16.7%), long-course chemotherapy + short-course radiation (1, 8.33%) or long-course chemotherapy (1/12, 8.33%).

Short-term results: The overall 30-d mortality was 2.08% (1 of 48 patients), 1 of 48 patients (2.08%) was discharged to a rehabilitation centre and 27 of 48 patients (56.3%) had some postoperative complication (all grades), with only one anastomotic leak (1/33 anastomoses, 5 protected, 3.03%). Major complications (Clavien–Dindo III–V) occurred in 7 of 48 patients (3.36%). No patient contracted postoperative SARS-CoV-2. Complications, mortality and the length of stay are presented in Table 4. The patient who died had moderate-to-severe dementia, underwent a Hartmann procedure and died of bronchoaspiration pneumonia.

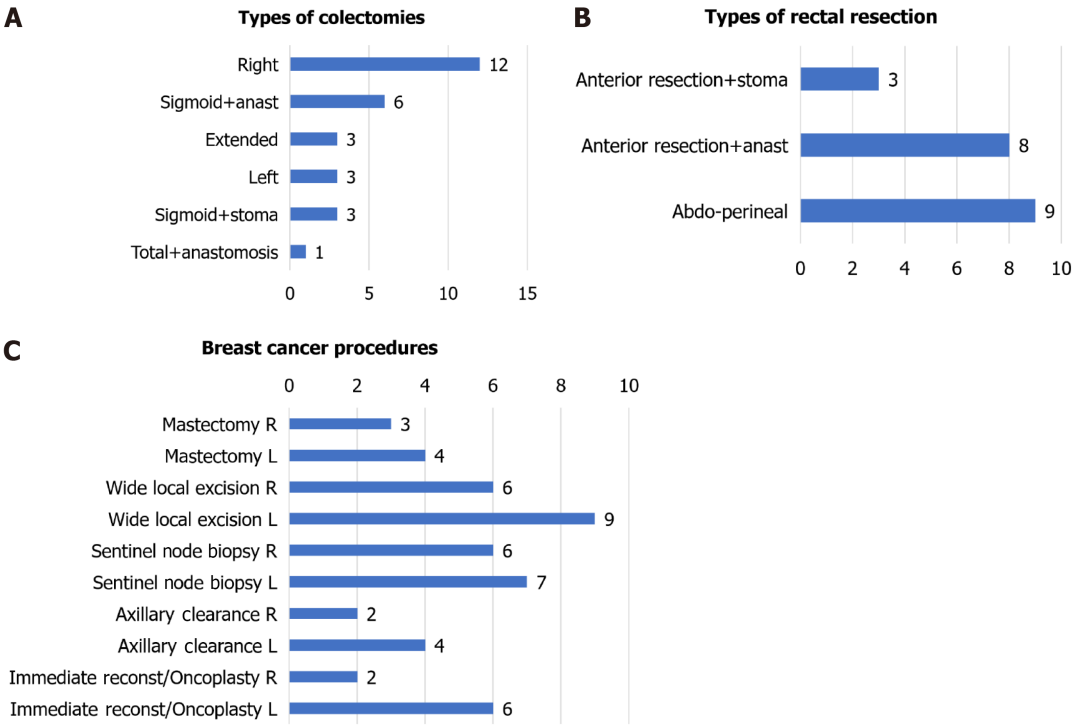

Figure 1 Surgical procedures performed. A: In patients with colon cancer; B: In patients with rectal cancer; C: In patients with breast cancer.

Changes relatable to pandemic in management: In the 29 patients with colon cancer who underwent surgery, in our opinion, 19 (65.5%) experienced a delay to surgery. Objectively, 9 of 22 patients scheduled for surgery (40.9%) had to wait more than 4 wk in surgical waiting list, including four who had to wait for more than 2 months. Twenty-three of 29 patients (79.3%) underwent surgery in an alternative building (COVID-19 free). One patient underwent a stoma at the beginning of the first wave, which seems very unlikely in the pre-COVID-19 context, and one surgeon changed his usual handsewn ileocolonic anastomosis to a mechanical one. In both cases, these choices were made to avoid possible complications requiring critical care, wishing to reduce hospital stay and/or based on the initial guidelines. There were three patients who underwent open surgery by general surgery consultants; some of these surgeries probably could have been laparoscopic without pandemic. Four patients did not have surgery during the first wave of the COVID-19 pandemic: Two died while awaiting surgery, one in hospital due to severe COVID-19, and the other rejected surgery and died at home with COVID-19. Another two patients refused surgery: One never underwent surgery (and died due to COVID-19 pneumonia in February 2021) and the other finally accepted surgery in August 2020.

All patients with rectal cancer underwent surgery. In our opinion, 13 of 20 patients (65.0%) suffered a delay to surgery. Objectively, 9 of 20 (45.0%) scheduled patients had to wait more than 4 wk on the waiting list, including four for more than 2 months, excluding window periods for long-course (8 wk in our hospital) or short-course (4 wk) radiotherapy, which were accomplished. Sixteen of 20 patients (80.0%) were operated on in the COVID-free building and two (10%) experienced delays after their diagnosis due to cancellation of outpatient clinics or endoscopic therapies. Twelve end stomas were performed, nine because of abdominoperineal resection, and five of eight anastomoses were protected with an ileostomy. In two cases the stoma was an intraoperative decision: one due to an adverse event (distal rectum perforation) and the other one attributable to the COVID-19 pandemic (to avoid possible complications requiring critical care).

Long-term follow-up: Concerning the oncological outcomes, the adjuvant therapies are schematised by disease stage in Table 5; the pre-pandemic indications were maintained. In the 28 patients with colon cancer, four patients with high-risk stage II and all stage III–IV suitable patients received chemotherapy (in total, 11/28, 39.29%). In the patients with rectal cancer, the patient with a complete remission was added (16/21, 76.19%). At the end of the follow-up, five patients with colon cancer (17.9%) and nine patients with rectal cancer (42.9%) had experienced a relapse. There were four cancer-related deaths (8.16%, two patients with colon cancer and two patients with rectal cancer), and another patient died due to COVID-19. For the patients with colon cancer who relapse, the mean DFS was 8.01 (range 1.56–18.5) months and in those who die (all causes), the mean OS was 16.66 (range 0.33–28.0) months. The 3-year DFS and OS were 82.1% and 78.6%, respectively. For the patients with rectal cancer, the mean DFS was 13.6 (range 2.07–36.0) months and the mean OS was 18.3 (range 7.63–34.5) months. The 3-year DFS and OS were 52.4% and 76.2%, respectively.

Regarding COVID-19, 42 of the 49 patients who underwent surgery (85.7%) got vaccinated between March and May 2021. Five patients were not vaccinated, and data for the other two were lost. Of the five unvaccinated, four died (not due to COVID-19) before they could get vaccinated; the reason why the other was not vaccinated is unknown. Sixteen of 49 patients (32.7%) contracted COVID-19 during the follow-up: nine after their vaccination, five before vaccination, one who did not get vaccinated and one whose vaccination data was lost. Only two of these 16 patients (12.5%) required hospital-

Table 1 Patient demographics and number of comorbidities by cancer type, *n* (%)

		Colorectal	HBP	OG	Breast
Total number		53 (100)	13 (100)	9 (100)	20 (100)
Age (yr)	40-49	3 (5.7)	2 (15.4)		5 (25.0)
	50-59	3 (5.7)	2 (15.4)		8 (40.0)
	60-69	13 (24.5)	5 (38.5)	5 (55.6)	3 (15.0)
	70-79	22 (41.5)	3 (23.1)	3 (33.3)	4 (20.0)
	80-89	11 (20.8)	1 (7.7)	1 (11.1)	
	90-99	1 (1.9)			
Sex	Female	18 (34)	7 (53.8)	2 (22.2)	19 (95.0)
	Male	35 (66)	6 (46.2)	7 (77.8)	1 (5.0)
ECOG perfor-mance score	0	13 (24.5)	5 (38.5)	3 (33.3)	19 (95.0)
	1	21 (39.6)	6 (46.2)	4 (44.4)	1 (5.0)
	2	17 (32.1)	2 (15.4)	2 (22.2)	
	3	2 (3.8)			
ASA	I	7 (13.2)	1 (7.7)	0	4 (20.0)
	II	25 (47.2)	6 (46.2)	6 (66.7)	13 (65.0)
	III	17 (32.1)	5 (38.5)	2 (22.2)	3 (15.0)
	IV	4 /7.5)	1 (7.7)	1 (11.1)	
BMI	< 18.5	0	1 (7.7)		
	18.5-24.9	10 (18.9)	4 (30.8)	3 (33.3)	7 (35.0)
	25-29.9	24 (45.3)	5 (38.5)	5 (55.6)	8 (40.0)
	30-34.9	15 (28.3)	1 (7.7)	1 (11.1)	1 (5.0)
	35-39.9	4 (28.3)	1 (7.7)		3 (15.0)
	≥ 40	0	1 (7.7)		1 (5.0)
Number of comorbidities	Mean (SD), range	3.13 (1.34) 1-6	2.85 (1.4), 1-5	3 (1.22) 1-5	1.85 (1.14) 0-4

ECOG: Eastern cooperative oncology group; ASA: American society of anesthesiology; BMI: Body mass index; HBP: Hepato-Bilio-Pancreatic; OG: Oesophago-Gastric.

Table 2 Patient comorbidities ordered by cancer type group

n (%)	CR, *n* = 53	HBP, *n* = 13	OG, *n* = 9	Breast, *n* = 16
Smoker	5 (9.4)	2 (15.4)	2 (22.2)	3 (18.8)
Chronic kidney disease (moderate/severe)	7 (13.2)	1 (7.7)	2 (22.2)	1 (6.3)
Chronic obstructive pulmonary disease	1 (1.9)	1 (7.7)	0	0
Asthma	1 (1.9)	1 (7.7)	1 (11.1)	0
Congenital abnormality - cardiac	1 (1.9)	0	0	0
Hypertension	29 (54.7)	5 (38.5)	6 (66.7)	7 (43.8)
Congestive heart failure	6 (11.3)	1 (7.7)	0	0
Ischemic heart disease	4 (7.5)	1 (7.7)	0	0
Peripheral vascular disease	2 (3.8)	0	0	0
Stroke/TIA	4 (7.5)	1 (7.7)	0	1 (6.3)

Dementia	1 (1.9)	0	0	
Diabetes mellitus	15 (28.3)	3 (23.1)	2 (22.2)	1 (6.3)
HIV infection	0	0	0	0
Others	39 (73.6)[1]	8 (61.5)[2]	6 (66.7)[3]	11 (68.8)[4]

[1]Atrial fibrilation (6 patients), Previous cancer (6), Cardiac valvular disease (4), Obstructive sleep apnea (3), Pulmonary hypertension (2), Pulmonary fibrosis (1), Liver cirrhosis with portal hypertension (1).
[2]Previous cancer (3), Pulmonary hypertension (1).
[3]Mitral insufficiency (1).
[4]Previous breast cancer (1), Previous colorectal and thyroid cancer (1).
CR: Colorectal; HBP: Hepato-Bilio-Pancreatic; OG: Oesophago-Gastric.

isation due to pneumonia (one before and other after vaccination); the last died due to bilateral pneumonia being cancer-free. 60% had asymptomatic or very oligosymptomatic clinical pictures.

The SARS-CoV-2-positive patient who underwent surgery did not have COVID-19 symptoms. He presented with T4aN1bM1 (liver) perforated superior rectal cancer with faecal diffuse peritonitis. He underwent the Hartmann procedure and had complicated recovery due to septic shock. He received adjuvant capecitabine and liver surgery (February 2021), but in May 2021, multiple liver metastases appeared. He died due to cancer progression 17 months after the index surgery. He was vaccinated in May 2021 and he did not became infected with SARS-CoV-2 again before his death.

Patients with liver and pancreatic cancer

There were seven patients with liver cancer and six with pancreatic cancer; 12 of them (92.3%) underwent surgery during the first wave of the pandemic. Their demographic data and comorbidities are described in Tables 1 and 2; all of them had at least one of the registered comorbidities (mean 2.85, SD 1.4, range 1–5). The cancer and surgical treatment characteristics are presented in Table 6.

In the six patients with pancreatic cancer, all the surgeries were elective, open and with a final curative intent. The following surgeries were performed: Left pancreatectomy (3/6, 50.0%; one with a total splenectomy), total pancreatectomy (2/6, 33.3%; one with a total splenectomy) and pancreaticoduodenectomy (1/5, 16.7%). None of these patients contracted COVID-19 during the first month after surgery and all were discharged home (length of stay: mean 8, SD 2.37, range 5–12 d).

All seven patients with liver cancer had colorectal metastases. One of them, scheduled for one-stage liver and colon surgery (preoperative T4N1 cancer), had his surgery cancelled and chemotherapy was resumed; in the re-staging he had developed bone metastases, so he never received surgery with a curative intent. All surgeries were elective and had a final curative intent. No patient received preoperative RFA/TACE. The following surgical procedures were performed: right hepatectomy (3/6, 50.0%; two with left-lobe metastasectomies and one with cholecystectomy), multiple metastasectomies (2/6, 33.3%) and laparoscopy-aided microwave ablation (1/6, 16.7%). No patient contracted COVID-19 and all were discharged home (length of stay: mean 6.33, SD 3.61, range 2–11 days).

Short-term results: The overall 30-d mortality was 0% and the complication rate (all grades) was 58.3%; it was higher among patients who underwent liver surgery (Table 7). Major complications (C-D grades III–V) occurred in 1 of 12 (8.33%); the highest complication grade was IIIA. Grade B/C PHLF affected 1 of 6 (16.7%) susceptible patients and there were no POPF.

Changes relatable to COVID-19 pandemic in management: For patients with pancreatic cancer, two (33.3%) had a delay, including one (16.7%) who was on the waiting list for more than 4 wk. Four of six patients (66.7%) were operated on in the alternative building, and one with a final R1 resection could not benefit from usual intraoperative margin status assessment. For patients with liver cancer, two (33.3%) had their planned surgery cancelled at the beginning of the study period. Chemotherapy was resumed in both; one progressed and never had surgery, and the other underwent surgery during the first wave. Four surgeries were delayed (66.7%, one for more than 4 wk), two patients (33.3%) underwent a longer course of neoadjuvant chemotherapy and two patients (33.3%) were operated on in an alternative hospital building.

Long-term follow-up: Concerning oncological outcomes, five of the 6 (83.3%) liver metastases patients who underwent surgery received adjuvant chemotherapy and all of them relapsed (five at a systemic location and one locally). The mean DFS was 8.32 (range 3.47–19.9) months. Four of these patients died (mean survival 22.12, range 14.4–36.07 months) due to cancer progression; the 3-year OS was 50%, and the 3.5-year OS was 33.3%. We will present the six patients with pancreatic cancer separated by histology. Concerning pancreatic carcinoma (Table 8), the 3-year DFS and OS were 33.3%. One patient was disease free at the end of the follow-up (this patient was also diagnosed of lung cancer in 2022), one died at 1.8 months due to pancreatitis and the other died at 14.3 months due to cancer. Both patients with neuroendocrine tumours were stage III and were disease free and alive at the end of the follow-up. The patient with distal cholangiocarcinoma was stage IIB, received adjuvant chemotherapy and radiotherapy, relapsed (DFS 14.6 months) and died due to cancer progression at 20.93 months.

Table 3 Characteristics of colorectal cancer and of the performed surgery

		Colon, *n* (%)	Rectal, *n* (%)
Total *n* (%)		32/28 operated	21 (1 positive)[3]
Final disease stage	0	1 (3.1)	1 (4.8)
	I	4 (12.5) + 2 (6.2)[1]	3 (14.3)
	IIA	6 (18.8) + 1 (3.1)[1]	7 (33.3)
	IIB	6 (18.8)	0
	IIIA	1 (3.1)	4 (19.0)
	IIIB	7 (21.7) + 1 (3.1)[2]	4 (19.0)
	IIIC	3 (9.4)	0
	IVA	0	2 (9.5)
Resection	R0	28 (100)	18 (90.0)
	R1	0	2 (10.0)
	R2	0	0
CRITCON level	0	13 (46.4)	9 (45.0)
	1	4 (14.3)	4 (20.0)
	2	8 (28.6)	4 (20.0)
	3	3 (10.7)	3 (15.0)
Urgency	Urgent/Expedited	4 (14.3)	0
	Elective	24 (85.7)	20 (100)
Operative approach	Open	10 (35.7)	1 (5.0)
	MIS	15 (53.6)	18 (90)
	MIS-open	3 (10.7)	1 (5.0)
Anastomosis	Yes, with protective stoma	0	5 (25.0)
	Yes	25 (89.3)	3 (15.0)
	No, end stoma only	3 (10.7)	12 (60.0)
Changes in anastomosis practice	No, typical practice	27 (96.4)	18 (90.0)
	Change unrelated to COVID	0	1 (5.0)
	Change due to pandemic	1 (3.6)	1 (5.0)

[1]Non operated patient.
[2]Non operated patient that became operated at August 2020.
[3]SARS-Cov-2 positive case only considered for staging.
MIS: Minimally invasive surgery; R0: No microscopic disease; R1: Microscopic disease; R2: Macroscopic disease at margin.

Table 4 Colorectal cancer patients' postoperative outcomes and stay

Colorectal cancer, *n* (%)	Right colon	Left colon	Rectal
Total, *n*	15	13	20
Mortality	0	1 (7.7)	0
Discharged rehabilitation center	0	1 (7.7)	0
Reoperation (CD III)	0	1 (7.7)	4 (20.0)
Unplanned critical care admission (CD IV)	0	0	1 (5.0) from theatre
Any complication	6 (40.0)	9 (69.2)	13 (65.0)

Anastomotic leak		0	0	1 (5.0)
Bleeding requiring transfusion		1 (6.7)	2 (15.4)	2 (10.0)
Ileus		3 (20.0)	0	5 (25.0)
Superficial/deep SSI		1 (6.7)	3 (23.1)	3 (15.0)
Organ/space SSI		0	1 (7.7)	2 (10.0)
Wound dehiscence		0	0	0
Acute kidney injury		4 (26.7)	3 (23.1)	5 (25.0)
Pneumonia		0	1 (7.7)	0
Other organ injury		0	0	1 (5.0)
Other		0	4 (30.8)	7 (35.0)
Length of stay (d)	mean (SD)	6.27 (2.76)	6.36 (1.91)	8.8 (7.36)

CD: Clavien Dindo classification; SSI: Surgical site infection.

Regarding COVID-19, 11 of the 13 patients (84.6%) got vaccinated between April and June 2021; the other two died, not due to COVID-19, before vaccination started. Five of the 13 patients (38.5%) contracted the infection during the follow-up after immunisation. Of these five patients, three (60%) were asymptomatic or mildly symptomatic, without hospital admissions, sequelae or death.

Patients with oesophago-gastric cancer

There were seven patients with gastric cancer and two with oesophageal cancer. Of them, seven (77.8%) underwent surgery in May and June, when the pandemic was more stabilized at our setting. Table 1 provides the patient demographic data. All of the patients had at least one comorbidity (mean 3, SD 1.22, range 1–5; Table 2). The cancer and surgery characteristics are presented in Table 9.

Of the seven patients with gastric cancer, five (71.4%) underwent an operation. One of these five patients (20.0%) had mild COVID-19 4 wk before surgery. All surgeries were elective except one (20.0%, due to obstruction) and had a definitive curative intent. The following surgical procedures were performed: total gastrectomy (2/5, 40.0%), atypical resections (2/5, 40.0%) and distal gastrectomy (1/5, 20.0%). Additional procedures included one feeding jejunostomy and one Dor fundoplication in a proximal atypical resection. No patient contracted COVID-19 during the first month after surgery and all were discharged home (length of stay: Mean 8.4, SD 3.13, range 5–13 days).

In the two patients with oesophageal cancer, the surgeries were elective and had a curative intent; neither patient had a previous SARS-CoV-2 infection. Both patients underwent oesophagogastrectomy, one with a total gastrectomy and one with a gastroplasty (three and two fields, respectively). One thorax was approached by thoracoscopy and the other by thoracotomy; both abdomens by open surgery. Neither patient contracted COVID-19 during the first month. One patient was discharged home but the other died on postoperative day 9 due to an anastomotic leak (length of stay: Mean 14, SD 7.77, range 9–19 days).

Short-term results: The overall 30-day mortality was 14.3% and complications (all grades) affected three of seven (42.9%), with the highest rate among patients with oesophageal cancer (Table 10). Major complications (C–D grades III–V) occurred in two of seven patients (28.6%).

Changes potentially relatable to COVID-19 pandemic in management: For patients with gastric cancer, two patients were not resected during the first wave. One contracted severe COVID-19 after the first neoadjuvant chemotherapy cycle and required hospitalisation. The MDT decision changed to surgery and when exploratory laparoscopy could be performed (17 June), peritoneal carcinomatosis was found. The other patient refused surgery and did not receive specific treatment but changed his mind in September 2020 and underwent surgery; an unresectable mass was found, and a palliative surgery was performed. For the five patients who underwent surgery, 3 (60.0%) took place in the COVID-free building, two (40%) had a delay [one patient with gastrointestinal stromal tumour (GIST) waited more than 4 wk on the waiting list] and one patient (20.0%, the first case operated) would likely had received a laparoscopic approach in the pre-COVID-19 setting. Concerning the two oesophageal cancer patients, both had a delayed surgery (more than 4 wk) and a very long period from the completion of neoadjuvant therapy to surgery. One patient (50.0%) was operated on in the alternative building. Perhaps without a health crisis, more minimally invasive approaches could have been intended.

Long-term follow-up

Concerning oncological outcomes and therapies, the three patients with gastric carcinoma who underwent surgery received adjuvant chemotherapy. One case with stage IIIC disease relapsed at 27.6 months (systemic) and died at 33.8 months due to cancer. The other two were disease free and alive at the end of the follow-up; thus, the 3-year DFS and OS were 66.7%. The patient with lymphoma received chemotherapy, had a systemic relapse at 5.83 months and died due to hemophagocytic syndrome at 7.27 months. The patient with GIST was disease free and alive at the end of the follow-up.

Table 5 Colorectal cancer patients' adjuvant therapies and oncological outcomes ordered by postoperative tumor-node-metastasis staging

Colorectal		Colon			Rectal			
		Chemo	Relapse	Death	Chemo	RT	Relapse	Death
I	No	4	3	2	2		2	3
	Yes	0	0	2 (PO/?)	1		1 (S)	
	Unknown	0	1					
IIA	No	5	6	5	3		4	6
	Yes	1	0	1 (COVID)	4		2 (L+R/L)	1 (Infection)
	Unknown						1	
IIB	No	3	5	5				
	Yes	3	1 (R)	1 (CA)				
IIIA	No	0	1	1	0		4	3
	Yes	1	0	0	4	1	0	1 (RAD)
IIIB	No	3	5	6	0		0	2
	Yes	4	2 (R+S/S)	1 (CA)	4		4 (S)	2 (CA/PO)
IIIC	No	1	1	2				
	Yes	2	2 (S)	1 (?)				
IVA	No	0	0	0	0		0	1
	Yes	0	0	0	2		2 (R+S/S)	1 (CA)
O	No	1	0	1	0		1	1
	Yes	0	0	0	1		0	

CA: Cancer; PO: Postoperative complication; ?: Unknown; RAD: Radiation enteritis; R: Regional; L: Local; S: Systemic; RT: Radiotherapy.

Table 6 Characteristics of liver and pancreatic cancer and performed surgeries

n (%)	Pancreas, *n* = 6		Liver, *n* = 6		
Histology	Adenocarcinoma	4 (66.7) [1DCC]	Tumour type	CR metastasis	6 (100.0)
	Neuroendocrine	2 (33.3)		Other	0
Resectability (NCCN classification)	Resectable	3 (50.0)	Pre-COVID Extent of resection	Minor	2 (33.3)
	Borderline resectable (vein)	2 (33.3)		Major	3 (50.0)
	Borderline resectable (artery)	0		Extra major	1 (16.7)
	Locally advanced	1 (16.7)			
Preoperative biliary drainage	0		0		
Previous COVID	0		0		
Neoadjuvant therapy	Long-course Chemo	1 (16.7)	4 (57.14)		
	Short-course Chemo		1 (14.3)		
	Radiochemotherapy	1 (16.7)			
CRITCON level	0	3 (50)	4 (66.7)		
	1	1 (16.7)	2 (33.3)		
	2	2 (33.3)			
Operative approach	Open	6 (100)	5 (83.3)		
	MIS		1 (16.7)		

Resection margin status	R0	3 (50.0)	4 (66.6)
	R1	3 (50.0) [2 NE]	1 (16.7)
	Imaging complete	0	1 (16.7)
Final staging	IB	2 (33.3)	
	IIB	2 (33.3) [1DCC]	
	III	2 (33.3) [2 NE]	

NE: Neuroendocrine tumour; DCC: Distal cholangiocarcinoma; Chemo: Chemotherapy; MIS: Minimally invasive surgery; R0: No microscopic disease at margin; R1: Microscopic disease at margin; R2: Macroscopic disease at margin.

The case of squamous cell oesophageal cancer died due to postoperative complications. Finally, the patient with stage IIIB oesophageal adenocarcinoma received no chemotherapy, relapsed at 4.67 months (local and regional) and died at 5.36 months due to bowel ischaemia.

Regarding COVID-19, four of the seven patients (57.1%) who underwent surgery were vaccinated between March and May 2021; the other three patients died before vaccination started. Two of the seven patients (28.6%) contracted COVID-19 during the follow-up: one after getting immunised and the other on an unknown date prior to vaccination (diagnosed as IgG+). One patient was asymptomatic and the other had a mild infection.

Patients with breast cancer

Twenty patients with breast cancer underwent elective surgery. The demographic data are described in Table 1. In contrast to the previous groups, 4 of the 20 patients (20.0%) had no comorbidities (mean 1.85, SD 1.14, range 0–4; Table 2). One patient had COVID-19 5 wk before undergoing surgery.

The cancer and surgical treatment characteristics are presented in Table 11. The principal surgical procedures performed were: partial breast excisions (15/20, 75.0%), total mastectomies (3/20, 15.0%) and skin-sparing mastectomy (2/20, 10.0%); two patients (10.0%) had bilateral mastectomies. The complete procedures (including associated axillae and reconstructive manoeuvres) are presented in Figure 1C. Except for one patient who had hormone-sensitive metastatic cancer for a long time, in whom mastectomy was indicated to control local symptoms, the intention of all surgeries was curative.

As expected, the short-term outcomes were favourable. No patient contracted COVID-19 during the 30-d postoperative period, and all of them were discharged home (length of stay: mean 1, SD 0.560, range 0–2 d), no patient required a re-intervention or ICU admission, and mortality was 0%. Five (25.0%) had a total of six mild complications (CD grade I): Three superficial surgical site infections and three seromas.

There were some changes potentially associated with the COVID-19 pandemic. Nineteen of the 20 patients (95%) were operated on in an alternative COVID-19-free building (including six in a private hospital) and twelve (60%) had to wait more than 4 wk on the waiting list. But the decision to perform oncoplastic surgery or a reconstruction was not modified in any case due to the pandemic.

Long-term follow-up

Concerning the oncological outcomes, the adjuvant therapies are schematised by disease stage in Table 8. Of the 20 patients, 12 (60.0%) received chemotherapy, 17 (85.0%) radiation and 15 (75.0%) hormone treatment, maintaining pre-pandemic indications. At the end of the follow-up, only 3 of the 20 patients (15.0%) had experienced a relapse. There were no cancer-related deaths and all patients were alive, with a 3-year DFS and OS of 85.0% and 100%, respectively.

Regarding COVID-19, 100% of the patients were vaccinated between February and June 2021. Eleven patients (55.0%) contracted COVID-19 during the follow-up, all after getting immunised. All were symptomatic being the most frequent cold symptoms (63.6%) and fever (9.0%). No patient required hospitalisation, had sequelae or died.

DISCUSSION

A specific board including hospital managers and physicians decided to resume cancer surgical care after analysing the evolution of the COVID-19 pandemic at our institution during the first weeks. Based on the available literature and recommendations during that period, a strategy based on limiting perioperative social contacts for patients (including avoiding family caregivers at the hospital), preoperative swab testing (48–72 h before surgery), establishing clearly defined COVID-19-free surgical pathways (including operating theatres, critical care areas and inpatient wards) and limiting infection among health professionals with the continued use of PPE was implemented. We believe that the physical structure of our hospital at that time, with two fully equipped separate buildings, facilitated establishing clean areas. In terms of postoperative SARS-CoV-2 infection, the strategy proved to be safe, as none of the patients who underwent surgery contracted COVID during the first postoperative month. Later, some of these measures were shown to not be sufficiently effective[27]; however, screening based on symptoms, preoperative testing (to identify carriers)[28] and clean areas[29] (including for urgent surgery[30]) were proved to be effective even for cancer surgery.

Table 7 Operated liver and pancreas cancer postoperative outcomes and stay

Pancreas and liver cancer, all results presented as *n* (%) unless specified			Pancreas, *n* = 6	Liver, *n* = 6
Overall complications	Mortality (CD V)		0 (0)	0 (0)
	Patients with complications		3 (50.0)	4 (66.7)
	Unplanned ICU admission (CD IV)		0	0
	Planned ICU admission		4 (66.7)	4 (66.7)
	Reintervention		1 (16.7) (III-A)	0
	Any major complication (CD III-V)		1 (16.7) III-A	0
	Acute kidney injury		2 (66.7)	2 (50.0)
	Ileus		1 (33.3)	1 (25.0)
	Bleeding requiring transfusion		0	1 (25.0)
	Superficial/Deep SSI		0	1 (25.0)
	Other		2 (66.7)[1]	0
HBP complications	Postoperative bleeding	No	6 (100)	6 (100)
	Bile leak	No	6 (100)	5 (83.3)
		Grade A	0	1 (16.7)
	Readmission with cholangitis		0	0
Pancreatic specific complications	Postoperative pancreatic fistula	No	6 (100)	-
Liver specific complications	Post-hepatectomy liver failure	No	-	3 (50.0)
		Grade A	-	2 (33.3)
		Grade B	-	1 (16.7)
		Grade C		0
	Length of stay (d)	Mean (SD)	8 (2.37)	6.33 (3.61)

[1]Gastric empty delaying and peripheral catheter infection.
HBP: Hepato-Bilio-Pancreatic; CD: Clavien-dindo; SSI: Surgical site infection; ICU: Intensive care unit.

Table 8 Pancreatic carcinoma and breast cancer adjuvant therapies and oncological outcomes by definitive tumor-node-metastasis staging

		Pancreas carcinoma			Breast			
		Chemo	Relapse	Death	Chemo	RT	HNT	Relapse
IA	No				5	1	2	9
	Yes				5	9	8	1 (S)
IB	No		1					
	Yes	2	1	2 (CA/PANC)				
IIA	No				0	1	0	3
	Yes				3	2	3	0
IIB	No		1	1				
	Yes	1		0				
IIIA	No							1
	Yes				1	1	1	0
IIIB	No				1	1	1	1
	Yes				1	1	1	1 (L)

IIIC	No				
	Yes				
IV	No				
	Yes	1	1	1	1 (R+S)
O	No	2	0	2	3
	Yes	1	3	1	0

CA: Cancer; PANC: Pancreatitis; R: Regional; L: Local; S: Systemic; RT: Radiotherapy; HNT: Hormone-therapy; Chemo: Chemotherapy.

Our 0% postoperative SARS-CoV-2 infection rate is quite notable. It is lower than in some of the CovidSurg-Cancer reports (3.8% for patients with colorectal cancer[31] and 6.2% for patients with liver and pancreatic cancer[32]). Very poor outcomes were reported initially in global studies with all type of surgical patients with perioperative SARS-CoV-2 infection, with mortality as high as 23.8%[9] or 19.8% in a meta-analysis of 2947 patients[33]. These results improved slightly in later publications with more selected populations. For example, mortality in patients with colorectal[31] or liver and pancreatic[32] cancer was 19.2% and 9.4%, respectively. In a comparative study from the United States (with 1:1 propensity-score matching to patients without COVID-19), mortality was 12% compared with 8.1% ($P < 0.001$) and postoperative complications, especially thromboembolic, were also higher[34].

The COVID-19 pandemic, especially the first waves, had a huge impact on cancer care: cancellation of consultations, screening and diagnostic tests and surgeries; medical material scarcity; fear to go to the hospital; and patients who contracted COVID-19, among others[35,36]. In our series, seven patients lost their opportunity to undergo surgery with a curative intent during the first wave. Two patients with colorectal cancer died due to COVID-19 before their surgery was scheduled. Two patients refused surgery: One never underwent surgery (died posteriorly due to COVID-19), and the other underwent surgery 4 months later without a clear impact on his oncological course. One patient with liver metastasis had his surgery cancelled and progressed (bone metastases) under chemotherapy; it is likely that his oncological course would not have been favourable even with liver surgery. In patients with gastric cancer, one patient contracted severe COVID-19 and could not undergo chemotherapy or surgery; when she recovered, peritoneal carcinomatosis was found. The other patient with gastric cancer refused surgery and when he changed his mind, an unresectable mass was found.

Other aspects could have impacted our patients. A high proportion (36/78, 46.2%) were on the surgical waiting list for more than 4 wk, considering that since their diagnosis some more weeks had passed. We used the date of waiting list inclusion to exclude the effect of the time for neoadjuvant therapies if indicated. Before the COVID-19 pandemic, although common sense dictated that surgical delay led to poorer outcomes, mostly for aggressive cancers, the available evidence was surprisingly scant, contradictory and difficult to decipher. This issue has been deeply analysed during this pandemic. Currently, we know that in patients with colorectal cancer, delays longer than 4 wk do not seem to affect complete resection rates or short-term outcomes (CovidSurg results)[37]. However, a meta-analysis confirmed than longer delays were significantly associated with worse OS (but not DFS) with a number needed to harm for a 1- and 3-month delay of 35 and 10, respectively[38]. A 2021 meta-analysis including 18 studies and 2533355 patients observed that delaying surgery for 12 wk decreased OS in breast cancer [hazard ratio (HR) 1.46, 95% confidence interval (CI) 1.28–1.65], lung cancer (HR: 1.04, 95%CI: 1.02–1.06) and colon cancer (HR: 1.24, 95%CI: 1.12–1.38)[39]. So, the COVID-19 pandemic likely negatively impacted the oncological outcomes of our cohort.

In addition, we had to make some modifications in our previous working methods: surgery in a different building and facility, continuous changes in all the staff, limitations in availability and changes in devices and instrumentation and new difficulties related to the use of PPE[15,16]. Moreover, at least at the beginning, there were continuous changes in the recommendations from scientific societies regarding surgical approaches, anastomoses, *etc*. Below, we discuss whether the combined effect of these aspects impacted the outcomes of our patients.

For colorectal cancer, apart from the treatment delays and changes in the location where the patients were treated, three patients with colon cancer were operated on by general surgery consultants (directly *via* open surgery), and we think that two definitive stomas were performed because of the pandemic situation. Our principal results are comparable with the outcomes published by the European Society of Coloproctology (ESCP) 2015 Right Hemicolectomy Audit[40], 2017 Left Colon, Sigmoid and Rectal Resections Audit[41,42] and elective colorectal cancer data from CovidSurg-Cancer [31], as presented in Table 12. The ESCP audits included contemporaneous data from more than 5000 patients from 54 countries. Although not significantly different from the rates published by the ESCP or CovidSurg, our rate of open surgery for colon cancer during the first wave of the COVID-19 pandemic was higher than usual in our institution (as an example in rectal cancer cohort laparoscopic rate is 90%). The anastomotic leak rate was 0% in patients with colon cancer but 12.5% (considering only performed anastomoses) in patients with rectal cancer. Moreover, mortality was < 2% for all types in both audits, but 7.7% for us in patients with left colon cancer (probably highly influenced by the low number of included patients). Concerning the long-term outcomes, we can compare our results with contemporary OS data obtained from the United States surveillance, epidemiology, and end results (SEER) program for cancer[43]. In 2019, the 3-year OS for colon cancer was 93.2%–93.5% (stages I–II), 78.9%–79.4% (stage III) and 23.2%–23.7% (stage IV). In our study, considering only deaths attributable to cancer, the 3-year OS was 93.7% for stages I–II and 90.9% for stage III. For rectal cancer, the 3-year OS in SEER was 91.9%–92.4% for stages I–II (100% in our study), 80.7%–81.4% for the regional stage

Table 9 Characteristics of gastric and oesophageal cancer and performed surgeries

Gastric and oesophageal cancer [*n* (%)]		Gastric, *n* = 7 (5 operated)	Oesophageal, *n* = 2
Final TNM staging	IB	1 (14.3)[1]	
	I-E	1 (14.3)[2]	
	IIA	2 (28.6), 1[3]	
	IIIA	1 (14.3)[3]	
	IIIB		2 (100)
	IIIC	2 (28.6)	
Location	Middle third		1 (50.0)
	Lower third – GEJ		1 (50.0)
	Proximal	2 (40.0)	
	Distal	3 (60.0)	
Histology	Adenocarcinoma	3 (60.0)	1 (50.0)
	Squamous cell		1 (50.0)
	Other	1 GIST (20.0) 1 lymphoma (20.0)	
Neoadjuvant therapy	Long-course RT+CT		2
	Long-course CT	2 (40%)	
Previous COVID		1 (20.0%)	0
CRITCON level	0	3 (60.0)	1 (50.0)
	1	1 (20.0)	1 (50.0)
	2	1 (20.0)	
Urgency	Urgent/Expedited		
	Elective		
Operative approach	Open	1 (20.0)	2 (100.0)
	MIS	3 (60.0)	0
	MIS-open	1 (20.0)	0
Resection	R0	5 (100)	1 (50.0)
	R1	0	1 (50.0)
	R2	0	0

[1]GIST patient.
[2]Lymphoma patient.
[3]1 non-operated patient.
GEJ: Gastro-oesophageal junction; RT: Radiotherapy; CT: Chemotherapy; MIS: Minimally invasive surgery; R0: No microscopic disease; R1: Microscopic disease; R2: Macroscopic disease at margin.

(80% in our study) and 27.9%–28.8% for stage IV (50% in our study).

For liver and pancreatic cancer, we are limited by a very small number of patients. Postoperative mortality was 0%, in line with recent national studies of unselected hospitals reporting in-hospital mortality rates after pancreatic surgery of 3.2%–8.6%[44,45] and 3.4%–5.8% for liver surgery[46,47], and with those reported from CovidSurg-Cancer (3.4% and 1.8%, respectively, for SARS-CoV-2-negative patients)[32]. Our incidence of major complications (8.33%) is also similar or even better than in CovidSurg-Cancer data (13.2%)[32]. Concerning the long-term oncological outcomes, our 3-year OS for patients with liver metastases (50.0%) is worse than in some population-based studies (approximately 70.0%)[48,49], but these results depend more on patient characteristics than in surgery itself. Our results in patients with pancreatic carcinoma (3-year OS 33.3%) are similar to those based on SEER database for stages Ib and IIb (20.0%–40.0%)[50].

Considering gastric and oesophageal cancer, the very low number of patients included limited our ability to compare the results. Both patients with oesophageal cancer had poor outcomes and major complications; one of them died. The poor outcomes are probably due to the high number of comorbidities (both were ASA III), advanced stage (both were IIB) and treatment delays. In the patients with gastric cancer, the results are more comparable with the standards (20%

Table 10 Operated gastric and oesophageal cancer patient's postoperative outcomes and hospital stay

Gastric and oesophageal cancer, all results presented as *n* (%) unless specified		Gastric, *n* = 5	Oesophagus, *n* = 2
Overall complications	Mortality (CD V)	0 (0)	1 (50.0)
	Patients with complications	1 (20.0)	2 (100.0)
	Unplanned critical care admission (CD IV)	0	0
	Planned ICU admission	2 (40.0)	2 (100.0)
	Reintervention (CD III)	0	1 (50.0) – IIIa (stent)
	Any major complication (CD III-V)	0	2 (100.0)
	Acute kidney injury		2 (100.0)
	Pulmonary embolism		1 (50.0)
	Septic shock		1 (50.0)
	Anastomotic leak		2 (100.0)
	Ileus		0
	Bleeding requiring transfusion	0	0
	Superficial/deep SSI	1 (20.0)	1 (50.0)
	Other	1 (20.0)[1]	1 (50.0)[2]
Length of stay (d)	Mean (SD)	8.4 (3.13)	14 (7.7)

[1]Acute gastric retention (GIST patient).
[2]Pleural effusion.
CD: Clavien-dindo; ICU: Intensive care unit; SSI: Surgical site infection.

complications, 0% major complications and 0% deaths) from population-based studies (17.1% major complications and 3.3% mortality)[51] or with cases operated on during 2020 in a Chinese hospital (5% and 0%, respectively)[52]. For gastric carcinoma, in the SEER database the 3-year OS is 74.3%–75.4% for stages I–II and 40%–41.2% for stage III. With only one stage IIA and two stage IIIC patients, our 3-year OS was 66.7%, slightly longer than in the SEER database[43].

As expected, the patients with breast cancer had fewer comorbidities, and we registered no mortality or major complications. Only 25.0% of the patients had mild complications that needed no invasive manoeuvres. We tried to maintain European quality indicators in breast cancer care[53] and the requirements of a specialised centre[54]. There were no relevant changes, other than some delays, in the surgeries or oncological therapies. Eighty per cent of the patients had localised disease (stage 0–II), which explains our excellent 3-year OS (100%) and DFS (85.0%), comparable to or better than the SEER database (for localised disease 99.7%–99.8%, for regional disease 91.1%–91.4% and for metastatic cases – one case in our series – 43.5%–44.3%)[43].

This study has several limitations. First, due to its merely descriptive nature, we did not accurately compare the results or waiting times with pre- or post-lockdown periods. We could only use the approximation of being on the surgical waiting list for less than or more than 4 wk, and we could not compare, for example, the rates of minimally invasive surgery or the outcomes. We were only able to compare with the standards or multinational audits. Second, the low number of included patients, especially for liver, pancreatic, gastric and oesophageal cancer, significantly limited our ability to draw definitive conclusions. Third, to understand the real impact of the first wave of the COVID-19 pandemic, we would need to have information about all patients who had the types of cancer we included. In the present study, we only included patients who were diagnosed and proposed for surgery.

CONCLUSION

Some patients lost their opportunity to undergo surgery with a curative intent due to the first wave of the COVID-19 pandemic. Our results reaffirm the idea that clearly established clean areas and pathways, even with physical barriers, were essential to continue treating patients with digestive and breast cancers and to minimise perioperative SARS-Cov-2 infection and its possible consequences. Although the practice recommendations and availability of PPE changed continuously, and we had to face some relevant alterations in our previous clinical practice, our department could offer a surgical management of digestive and breast cancers with minimal changes compared with the pre-COVID-19 pandemic period, and with outcomes comparable to the standards.

Table 11 Characteristics of breast cancer and performed surgeries

All results presented as *n* (%) unless specified		Breast, *n* = 20
Postoperative TNM staging	0	3 (15.0)
	IA	10 (50.0)
	IIA	3 (15.0)
	IIIA	1 (5.0)
	IIIB	2 (10.0)
	IV	1 (5.0)
Histology	Invasive carcinoma	19 (95.0)
	Ductal carcinoma in situ (high grade)	1 (5.0)
	Estrogen receptor (+/-)	15/5
	Progesteron receptor	11/9
	Her-2 (+/-)	3/16
	Nottingham prognostic index (*N*=19). [Mean/SD/Range]	3.65/1.11/ 2 - 6.60
	Oncotype score (*N*=7). [Mean/SD/Range]	48.86/23.53/10 - 80
Menopausal status[1]	Post/Pre-menopausal	15 (75.0)/4 (20.0)
Neoadjuvant therapies	Long-course chemotherapy	3 (15.0)
Previous COVID		1 (5.0)
CRITCON level	1	2 (10.0)
	2	10 (50.0)
	3	8 (40.0)
Resection	R0	17 (85.0)
	R1	2 (10.0)
	R2	1 (5.0)

[1]1 patient not valuable (man).
R0: No microscopic disease; R1: Microscopic disease; R2: Macroscopic disease at margin; TNM: Tumor-node-metastasis.

Table 12 Comparison of colorectal cancer outcomes with the European Society of Coloproctology international audit and CovidSurg collaboration data

	Right colon			Left colon			Rectal		
%	ESCP	CSURG	Ours	ESCP	CSURG	Ours	ESCP	CSURG	Ours
N	2225	724	15	989	367	13	2579	935	20
MIS	54.4	54.7	53.3	53.6	62.9	53.8	54.2	57.8	90
Open	36.5	41	33.3	36.8	29.7	38.4	35.8	38.0	5
Conv	9.1	4.3	13.3	9.6	7.4	7.7	10	4.2	5
ANAST	98.6	93.5	100	93.3	86.1	76.9	42.8	37.4	27.3
ANAST +DEF	0.3	1.4	0	1.8	5.7	0	33.5	35.4	45.45[1]
End stoma	1.1	5.1	0	4.9	8.2	23.1	23.7	27.2	27.3[1]
Leak	6.5	3.6	0	7.5	4.1	0	9.2	6.5	5; 12.5[2]
Death	1.7	1.2	0	0.7	1.6	7.7	0.8	2.0	0

[1]Excluded abdominoperineal resection patients.
[2]Considering only anastomosed patients. Data shown as % except first file.
1 patient with total colectomy in included in left colon (sigmoid cancer). MIS: Minimally invasive surgery; Conv: MIS-Conversion; ANAST: Anastomoses; DEF: Defunctioning stoma; ESCP: European society of coloproctology; CSURG: CovidSurg cancer study on colorectal surgery.

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FOOTNOTES

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Country of origin: Spain

ORCID number: Jacobo Trébol 0000-0002-6579-533X; Ana Carabias-Orgaz 0000-0001-8420-0242; María Carmen Esteban-Velasco 0000-0002-5055-7371; Asunción García-Plaza 0000-0002-1150-7937; Juan Ignacio González-Muñoz 0000-0002-6266-9055; Ana Belen Sánchez-Casado 0000-0001-9690-0131; Felipe Carlos Parreño-Manchado 0000-0001-8133-5627; Marta Eguía-Larrea 0000-0003-3953-685X; José Antonio Alcázar-Montero 0000-0001-7876-448X.

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ORIGINAL ARTICLE

Randomized Clinical Trial

Is there a correlation between the changes in airway inflammation and the changes in respiratory mechanics after vaping in patients with asthma?

Serafeim-Chrysovalantis Kotoulas, Kalliopi Domvri, Alexandros Tsantos, Ioanna Papagiouvanni, Anastasia Michailidou, Dionisios G Spyratos, Konstantinos Porpodis, Ioanna Grigoriou, Despina Papakosta, Athanasia Pataka

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Serafeim-Chrysovalantis Kotoulas, Intensive Care Unit, Hippokration General Hospital, Thessaloniki 54642, Greece

Kalliopi Domvri, Pulmonary Department-Oncology Unit, Aristotle University of Thessaloniki, Thessaloniki 57010, Greece

Alexandros Tsantos, 2nd Department of Internal Medicine, General Hospital of Thessaloniki ''Ippokration'', Thessaloniki 54642, Greece

Ioanna Papagiouvanni, Fourth Department of Internal Medicine, Aristotle University of Thessaloniki, Thessaloniki 54642, Thessaloniki, Greece

Anastasia Michailidou, 2nd Propaedeutic Department of Internal Medicine, General Hospital of Thessaloniki "Ippokration", Thessaloniki 54642, Greece

Dionisios G Spyratos, Konstantinos Porpodis, Despina Papakosta, Department of Pulmonary, Aristotle University of Thessaloniki, Thessaloniki 57001, Greece

Ioanna Grigoriou, Respiratory Failure Clinic, Papanikolaou General Hospital, Thessloniki 57001, Greece

Athanasia Pataka, Department of Respiratory Medicine, Aristotle Univ Thessaloniki, Sch Med, G Papanikolaou Hosp, Resp Failure Unit, Aristotle University of Thessaloniki, Thessaloniki 57001, Greece

Corresponding author: Serafeim-Chrysovalantis Kotoulas, PhD, Doctor, Intensive Care Unit, Hippokration General Hospital, Kostantinoupoleos 49, Thessaloniki 54642, Greece. akiskotoulas@hotmail.com

Abstract

BACKGROUND

Electronic cigarettes (ECs) have been promoted as alternatives to traditional cigarettes.

AIM
To investigate ECs' effects on respiratory system, especially in patients with respiratory diseases.

METHODS
We randomly selected 25 smokers with stable moderate asthma and matched them with 25 healthy smokers. All were subjucted to pulmonary function tests (PFTs), impulse oscillometry (IOS), fraction exhaled Nitric Oxide (FeNO), exhaled breathe condensate (EBC) and biomarker measurements before and after vaping one nicotine-containing EC.

RESULTS
The increase in FeNO 30 minutes after EC, reflecting airway inflammation, significantly correlated with increase of residual volume (RV), total lung capacity, respiratory impedance at 5 Hz (Z5Hz) and respiratory resistance at 5 and 20 Hz (R5Hz and R20Hz). No significant correlations were found between EBC biomarkers' changes and respiratory mechanics.

CONCLUSION
This is the first study demonstrating that the changes in airway inflammation caused by EC have direct effects in respiratory mechanics of asthmatic patients.

Key Words: Asthma; Electronic cigarette; Airway inflammation; Lung function; Impulse oscillometry; FeNO

Core Tip: This is the first study that correlates the acute changes in pulmonary function and in airway inflammation in patients with asthma after vaping one electronic cigarette.

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INTRODUCTION

Electronic cigarette (EC) is a novel smoking product promoted to replace tobacco cigarette in smokers' daily habits. However, despite the fact that the effects of the tobacco cigarette have been investigated thoroughly the last decades, this is not the case for EC. There is evidence that EC have several acute effects on lung function of healthy individuals[1-6], and on lung function and airway inflammation of patients with asthma[4-6] and chronic obstructive pulmonary disease (COPD)[6,7]. In a previous study, we have evaluated the acute effects of EC on lung function and airway inflammation in patients with asthma compared to healthy individuals[4]. However, we did not evaluate the possible correlations of the changes on airway inflammation with the acute effects of EC on lung function.

With this study we investigate the correlations between the changes in airway inflammation, reflected by the changes in fraction exhaled Nitric Oxide (FeNO) and exhaled breathe condensate (EBC) biomarkers, with the changes in pulmonary function tests (PFTs) and impulse oscillometry after vaping one nicotine-containing EC.

MATERIALS AND METHODS

Ethical approval

The protocol of this study was approved by the Ethics Committee of the Medical School, Aristotle University of Thessaloniki, reference 369-8/22.2.2017, before the initiation of enrolment and all participants gave their written informed consent. The protocol of the study was also registered in ISRCTN-registry (ISRCTN89151172).

Data collection process

The participants in this study[4] were divided into two groups: (1) The "asthmatic group" which consisted of asthmatic patients with moderate persistent stable asthma well controlled by receiving "step 3" treatment according to global initiative for asthma (GINA) guidelines[8]; and (2) the "control group" which consisted of healthy individuals. The participants of both groups were current every day smokers[9]. Exclusion criteria for the asthmatic group were: (1) Age under 18 years; (2) an acute asthma exacerbation the last month before enrollment; (3) a change in the asthma medication

the last month before enrollment; and (4) another acute or chronic disease apart from asthma the last two weeks before enrollment. Out of 87 eligible patients, 25 were recruited by using a random number generator. Exclusion criteria for the control group were: (1) Age under 18 years; and (2) the existence of any acute or chronic disease the last two weeks before enrollment. The recruitment of the 25 participants of the control group was made after matching them with those of the asthmatic group for gender, age, body mass index and smoking history (number of packyears).

Methodology

All participants were subjected to PFTs (spirometry, static lung volumes and Diffusion Lung Capacity for Carbon Monoxide)[10], total respiratory resistances measurement with an impulse oscillometry system (IOS)[11], FeNO measurement[12] and EBC gathering[13] for measurement of pH[13] and of concentrations of Interleukins 1b, 4, 5, 6, 8, 10, 13 and 17A and tumor necrosis factor-alpha with flow cytometry and of concentrations of 8-Isoprostane and leukotriene B4 with enzyme-linked immunosorbent assay[13]. Subsequently, participants vaped an EC, which had the same concentration of nicotine in the cartridge (medium nicotine content) and was of the same company. The composition of the e-liquid had been analyzed in a previous study[1]. Participants of both groups vaped the EC for five minutes (10 puffs with 30-second intervals between puffs). A new cartridge and atomiser were used for every participant. During EC inhalation two asthmatic patients presented mild cough and wheezing, which resolved automatically after a few minutes without necessitate reliever medication usage. Fifteen minutes after vaping the participants were subjected again to PFTs and IOS measurements and 30 min after vaping they were subjected to a new FeNO measurement and EBC gathering.

Data monitoring

Statistical analysis was performed using the SPSS software. All variables were continuous. To investigate for correlations between the changes in airway inflammation and the changes in respiratory mechanics linear regression was used between the difference in FeNO and in EBC biomarkers and the difference in PFTs and in IOS before and after the vaping of the EC in both groups.

RESULTS

There were no significant correlations between the changes in FeNO and the changes in respiratory mechanics before and after EC in the control group (Table 1). On the other hand, the difference in FeNO before and after EC in the asthmatics was significantly correlated with the corresponding differences in residual volume (RV), total lung capacity and respiratory impedance at 5 Hz (Z5Hz) (Table 1). Furthermore, the difference in FeNO before and after EC in the asthmatics was correlated with the corresponding differences in respiratory resistance at 5 and 20 Hz (R5Hz and R20Hz) at the limit of significance, where the number 0.000 was the lowest limit of the 95% of the confidence intervals (Table 1). However no significant correlations were found between the changes in the EBC biomarkers and the changes in respiratory mechanics before and after EC neither in the asthmatic nor in the control group.

DISCUSSION

FeNO has been associated with Th2 regulated asthma, reflecting eosinophilic inflammation[14]. Z5Hz is increased in worsening asthma[4,5], and the same applies for both R20Hz and R5Hz which reflect large and total airway resistance respectively[4,5]. The association between the changes in FeNO and in Z5Hz, R5Hz and R20Hz in the asthmatics after vaping could be attributed to the aggravation of airway inflammation in these patients (increase of FeNO), leading to bronchoconstriction (increase in impulse oscillometry indices), something that was not the case for the control group.

The increase of RV and TLC indicates the existence of air trapping and hyperinflation in obstructive lung diseases[15,16]. The correlation between the difference in FeNO before and after EC and the corresponding differences in RV and TLC in the asthmatic patients means that the deterioration of the airway inflammation is significantly associated with air trapping and hyperinflation, something not observed in the control group. This is particularly interesting since these defects are not as common in asthma as in COPD and when they are observed in asthma, they are associated with longer disease duration and fixed airflow obstruction[17,18].

Study limitations

The main limitation of this study is the relatively small number of participants in each group. Perhaps, this is the reason why the correlation between the difference in FeNO and the difference in R5Hz and R20Hz were at the limit of significance and there was no statistically significant correlation between the difference in FeNO and the differences in other key asthma indices such as forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), FEV1/FVC, peak expiratory flow and forced expiratory flow between 25% and 75% of FVC (FEF25-75). Possibly for the same reason there were no significant correlations between the changes in the EBC biomarkers and the changes in respiratory mechanics. Additional limitations of the study are that the measurements during the follow up were assessed only in one timepoint after EC, and only a specific brand of EC was used. Asthma is a chronic inflammatory airway disease that requires a long period of time for treatment and evaluation. Evaluating only the changes in lung function related indices or markers after a single inhalation of EC might not illustrate the effect of EC on asthma lung function. Additionally, only well controlled asthmatic patients of GINA "step-3" were evaluated. All the asthmatic patients studied were receiving treatment with

Table 1 Linear regression between the difference in fraction exhaled Nitric Oxide (particles per billion) and the difference in pulmonary function tests and in impulse oscillometry before and after the vaping of one electronic cigarette

Conrol group (*n* = 25)			
Variable	**Unstandardized coefficients (B) (95%CI)**	**Standardised coefficients (*r*)**	***P* value**
diff_FEV1 (L)	0.004 (-0.009–0.016)	0.131	0.53
diff_FVC (L)	-0.004 (-0.021–0.014)	-0.089	0.67
diff_FEV1/FVC (%)	0.096 (-0.216–0.407)	0.131	0.53
diff_PEF (L/s)	-0.065 (-0.166–0.035)	-0.269	0.19
diff_FEF25-75 (L/s)	0.000 (-0.053–0.054)	0.004	0.99
diff_RV (L)	0.010 (-0.005–0.025)	0.281	0.17
diff_TLC (L)	0.001 (-0.018–0.019)	0.015	0.94
diff_Z5Hz (kPa/L/s)	0.000 (-0.006–0.006)	-0.012	0.96
diff_R5Hz (kPa/L/s)	-0.001 (-0.007–0.004)	-0.112	0.59
diff_R20Hz (kPa/L/s)	-0.001 (-0.006–0.000)	-0.044	0.84
Asthma group (*N* = 25)			
diff_FEV1 (L)	-0.007 (-0.016–0.002)	-0.327	0.11
diff_FVC (L)	0.005 (-0.005–0.015)	0.203	0.33
diff_FEV1/FVC (%)	-0.027 (-0.173–0.119)	-0.080	0.71
diff_PEF (L/sec)	-0.010 (-0.087–0.066)	-0.059	0.78
diff_FEF25-75 (L/s)	-0.007 (-0.033–0.020)	-0.110	0.60
diff_RV (L)	0.027 (0.005–0.049)	0.465	0.019
diff_TLC (L)	0.018 (0.005–0.031)	0.519	0.008
diff_Z5Hz (kPa/L/s)	0.005 (0.000–0.010)	0.402	0.046
diff_R5Hz (kPa/L/s)	0.005 (0.000–0.010)	0.386	0.057
diff_R20Hz (kPa/L/s)	0.003 (0.000–0.006)	0.358	0.078

FeNO: Fractional exhaled Nitric Oxide; ppb: Particles per billion; EC: Electronic cigarette; N: Number; CI: Confidence intervals; diff: Difference; FEV1: Forced expiratory volume in 1 second; L: Liters; FVC: Forced vital capacity; PEF: Peak expiratory flow; sec: Second; FEF25-75: Forced expiratory flow between 25% and 75% of FVC; RV: Residual volume; TLC: Total lung capacity; Z5Hz: Respiratory impedance at 5 Hz; Hz: Hertz; kPa: Kilopascal, R5Hz; R20Hz: Respiratory resistance at 5, 20 Hz.

inhaled corticosteroids (ICS) and it is possible that the presence of ICS could have implicated with the changes in lung function and airway inflammation[19,20], after the use of the EC.

CONCLUSION

In conclusion, this study demonstrates that there is a direct association between airway inflammation and respiratory mechanics in patients with asthma after the use of EC, even in patients receiving ICS. As EC becomes more popular, there is an urgent need for studies which will assess its effects in human health, especially in certain group of respiratory patients as those with asthma.

ARTICLE HIGHLIGHTS

Research background

Electronic cigarette (EC) is a novel smoking product promoted to replace tobacco cigarette in smokers' daily habits. However, despite the fact that the effects of the tobacco cigarette have been investigated thoroughly the last decades, this is not the case for EC. There is evidence that EC have several acute effects on lung function of healthy individuals, and on

lung function and airway inflammation of patients with asthma and chronic obstructive pulmonary disease. In a previous study, we have evaluated the acute effects of EC on lung function and airway inflammation in patients with asthma compared to healthy individuals. However, we did not evaluate the possible correlations of the changes on airway inflammation with the acute effects of EC on lung function.

Research motivation

With this study we investigate the correlations between the changes in airway inflammation, reflected by the changes in fraction exhaled Nitric Oxide (FeNO) and exhaled breathe condensate (EBC) biomarkers, with the changes in pulmonary function tests (PFTs) and impulse oscillometry after vaping one nicotine-containing EC.

Research objectives

With this study we investigate the correlations between the changes in airway inflammation, reflected by the changes in FeNO and EBC biomarkers, with the changes in PFTs and impulse oscillometry after vaping one nicotine-containing EC.

Research methods

The protocol of this study was approved by the Ethics Committee of the Medical School, Aristotle University of Thessaloniki, reference 369-8/22.2.2017, before the initiation of enrolment and all participants gave their written informed consent. The protocol of the study was also registered in ISRCTN-registry (ISRCTN89151172).

Research results

There were no significant correlations between the changes in FeNO and the changes in respiratory mechanics before and after EC in the control group. On the other hand, the difference in FeNO before and after EC in the asthmatics was significantly correlated with the corresponding differences in residual volume, total lung capacity and respiratory impedance at 5 Hz. Furthermore, the difference in FeNO before and after EC in the asthmatics was correlated with the corresponding differences in respiratory resistance at 5 and 20 Hz (R5Hz and R20Hz) at the limit of significance, where the number 0.000 was the lowest limit of the 95% of the confidence intervals. However no significant correlations were found between the changes in the EBC biomarkers and the changes in respiratory mechanics before and after EC neither in the asthmatic nor in the control group.

Research conclusions

In conclusion, this study demonstrates that there is a direct association between airway inflammation and respiratory mechanics in patients with asthma after the use of EC, even in patients receiving inhaled corticosteroids.

Research perspectives

As EC becomes more popular, there is an urgent need for studies which will assess its effects in human health, especially in certain group of respiratory patients as those with asthma.

FOOTNOTES

Author contributions: Kotoulas SC, Spyratos DG, Porpodis K, Papakosta D, and Pataka A designed the research; Kotoulas SC, Domvri K, Tsantos A, Papagiouvanni I, Michailidou A, and Grigoriou I performed the research; Domvri K contributed new reagents or analytic tools; Kotoulas SC analyzed the data; Kotoulas SC, Domvri K, Tsantos A, Papagiouvanni I, Michailidou A, Spyratos DG, Porpodis K, Grigoriou I, Papakosta D, and Pataka A wrote the paper.

Institutional review board statement: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The protocol of the study was approved by the Ethics Committee of the Medical School, Aristotle University of Thessaloniki, reference 369-8/22.2.2017, before the initiation of enrolment. The protocol of the study was also registered in ISRCTN-registry (ISRCTN89151172).

Clinical trial registration statement: This study is registered at ISRCTN Clinical Trial Registry. The registration identification number is ISRCTN89151172.

Informed consent statement: All participants gave their informed written consent to participate in the study.

Conflict-of-interest statement: All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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Country/Territory of origin: Greece

ORCID number: Serafeim-Chrysovalantis Kotoulas 0000-0003-6092-1341; Ioanna Papagiouvanni 0000-0001-5190-0154.

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SYSTEMATIC REVIEWS

Non-enzymatic methods for isolation of stromal vascular fraction and adipose-derived stem cells: A systematic review

Vamsi Krishna Mundluru, MJ Naidu, Ravi Teja Mundluru, Naveen Jeyaraman, Sathish Muthu, Swaminathan Ramasubramanian, Madhan Jeyaraman

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Vamsi Krishna Mundluru, MJ Naidu, Ravi Teja Mundluru, Department of Orthopaedics, MJ Naidu Super Speciality Hospital, Vijayawada 520002, Andhra Pradesh, India

Vamsi Krishna Mundluru, MJ Naidu, Ravi Teja Mundluru, Naveen Jeyaraman, Madhan Jeyaraman, Department of Regenerative Medicine, StemC Clinics, Vijayawada 520002, Andhra Pradesh, India

Naveen Jeyaraman, Madhan Jeyaraman, Department of Orthopaedics, ACS Medical College and Hospital, Dr. MGR Educational and Research Institute, Chennai 600077, Tamil Nadu, India

Naveen Jeyaraman, Sathish Muthu, Madhan Jeyaraman, Department of Orthopaedics, Orthopaedic Research Group, Coimbatore 641045, Tamil Nadu, India

Sathish Muthu, Department of Orthopaedics, Government Medical College and Hospital, Karur 639004, Tamil Nadu, India

Sathish Muthu, Department of Biotechnology, Faculty of Engineering, Karpagam Academy of Higher Education, Coimbatore 641021, Tamil Nadu, India

Swaminathan Ramasubramanian, Department of Orthopaedics, Government Medical College, Omandurar Government Estate, Chennai 600002, Tamil Nadu, India

Corresponding author: Madhan Jeyaraman, MS, PhD, Assistant Professor, Research Associate, Department of Orthopaedics, ACS Medical College and Hospital, Dr. MGR Educational and Research Institute, Velappanchavadi, Chennai 600077, Tamil Nadu, India. madhanjeyaraman@gmail.com

Abstract

BACKGROUND

Adipose-derived stem cells (ADSCs) and the stromal vascular fraction (SVF) have garnered substantial interest in regenerative medicine due to their potential to treat a wide range of conditions. Traditional enzymatic methods for isolating these cells face challenges such as high costs, lengthy processing time, and regulatory complexities.

AIM

This systematic review aimed to assess the efficacy and practicality of non-enzymatic, mechanical methods for isolating SVF and ADSCs, comparing these to

conventional enzymatic approaches.

METHODS
Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines, a comprehensive literature search was conducted across multiple databases. Studies were selected based on inclusion criteria focused on non-enzymatic isolation methods for SVF and ADSCs from adipose tissue. The risk of bias was assessed, and a qualitative synthesis of findings was performed due to the methodological heterogeneity of the included studies.

RESULTS
Nineteen studies met the inclusion criteria, highlighting various mechanical techniques such as centrifugation, vortexing, and ultrasonic cavitation. The review identified significant variability in cell yield and viability, and the integrity of isolated cells across different non-enzymatic methods compared to enzymatic procedures. Despite some advantages of mechanical methods, including reduced processing time and avoidance of enzymatic reagents, the evidence suggests a need for optimization to match the cell quality and therapeutic efficacy achievable with enzymatic isolation.

CONCLUSION
Non-enzymatic, mechanical methods offer a promising alternative to enzymatic isolation of SVF and ADSCs, potentially simplifying the isolation process and reducing regulatory hurdles. However, further research is necessary to standardize these techniques and ensure consistent, high-quality cell yields for clinical applications. The development of efficient, safe, and reproducible non-enzymatic isolation methods could significantly advance the field of regenerative medicine.

Key Words: Adipose-derived stem cells; Stromal vascular fraction; Regenerative medicine; Non-enzymatic isolation; Mechanical separation techniques

Core Tip: This study highlights the superiority of non-enzymatic methods as alternatives for the isolation of stromal vascular fraction from adipose tissue. It emphasizes the necessity of standardizing these methods to ensure the procurement of consistent and high-quality cell yields suitable for a range of clinical applications.

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INTRODUCTION

Regenerative medicine has emerged as a pivotal area of interest across multiple medical specialties, driven by an increasing volume of literature on the potential of regenerative cells for a myriad of indications. Among various sources, adipose tissue is recognized for its significant role beyond shock absorption, thermoregulation, and energy storage; it stands out as the largest and most crucial reservoir for adipose-derived stem or stromal cells (ADSCs). These cells are predominantly found within the perivascular region of the stroma, an area characterized by a loose connective tissue matrix that houses a diverse array of cells including immune cells, erythrocytes, mesenchymal stem cells (MSCs), and other stromal components[1-4]. The ease of collection through liposuction, a minimally invasive procedure performed under local anesthesia, further underscores the accessibility of adipose tissue for regenerative therapies. Historically, the therapeutic potential of adipose tissue dates back to World War I, when Morestin first utilized fatty tissue injections to enhance wound healing in soldiers. This early application laid the groundwork for the field, which gained substantial momentum following the work of Zuk *et al*[5], who highlighted adipose tissue as a prime source of MSCs[5]. Recent studies have delved into the capabilities of ADSCs, particularly those within the stromal vascular fraction (SVF), focusing on their role in tissue regeneration for injuries and chronic conditions[6,7]. The SVF's rich secretome and the multipotent nature of its cellular constituents underscore its therapeutic potential[8,9].

However, the conventional method of isolating ADSCs from adipose tissue, primarily through enzymatic dissociation, poses significant challenges, including operational complexity and the need for specialized equipment, rendering it impractical for immediate surgical application[10,11]. This enzymatic process, despite its efficacy in isolating SVFs, disrupts the stem cell niche and necessitates compliance with good manufacturing practice standards, as defined by regulatory authorities[12,13]. Such limitations have catalyzed interest in mechanical stromal-cell separation techniques,

exemplified by the development of nanofat by Tonnard *et al*[2], which offers a non-enzymatic alternative for cell isolation. Despite the advent of intraoperative isolation techniques that promise to circumvent the challenges of enzymatic methods, there remains a paucity of research comparing the efficacy, cell yield, and phenotype of cells isolated through these novel mechanical methods to the traditional enzymatic approach[11,14]. This knowledge gap is particularly significant given the logistical and operational constraints faced by peripheral hospitals, which often lack the resources for the labor-intensive enzymatic isolation of ADSCs[15].

This systematic review aimed to critically assess the therapeutic potential of non-enzymatic methods for producing SVF, comparing these newer mechanical isolation techniques against the established enzymatic method. By evaluating the quality and quantity of SVF obtained through non-enzymatic methods, this review seeks to address a critical gap in the literature and validate the feasibility of these approaches for regenerative medicine applications.

MATERIALS AND METHODS

This systematic review was meticulously designed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, ensuring a structured and transparent methodology. The PRISMA flow diagram, illustrating the search and selection process, is presented in Figure 1[16]. The foundation of our search strategy was the well-established Population, Intervention, Comparison, and Outcome framework[17], which facilitated a focused and comprehensive literature search. It is noteworthy to mention that this investigation did not undergo formal registration, as it was developed primarily for academic purposes, specifically as part of a master's thesis project.

Eligibility criteria

The selection of studies for inclusion in this systematic review was governed by the precise inclusion and exclusion criteria tailored to the objectives of our investigation.

Inclusion criteria: Our review targeted studies that discussed non-enzymatic isolation procedures for the isolation of the SVF from adipose tissue. We included studies that utilized the adipose fraction obtained from lipoaspirate and those that evaluated the effectiveness of centrifugation forces, sonication, or red blood cell (RBC) lysis buffer. Studies were also considered if they compared non-enzymatic isolation techniques directly with enzymatic methods.

Exclusion criteria: We excluded studies published before the year 2000 and those not written in English to maintain a contemporary focus and ensure comprehension across the research team. Articles that solely utilized enzymatic isolation techniques or combined enzymatic with mechanical methods for SVF extraction were not considered. Furthermore, case studies, case series, and reviews focusing exclusively on adipose tissue processing techniques for fat grafting purposes were disregarded to maintain a clear focus on SVF isolation methodologies.

Information sources and search strategy

A comprehensive search was conducted across several databases, including The Cochrane Central Register of Controlled Trials, Embase (OvidSP), and PubMed, to identify relevant studies. The search strategy was meticulously crafted, combining keywords and phrases related to the population of interest (adipose stromal cells, ADSCs, adipose stem cells, stem cells, and SVF) with terms associated with the intervention (cell separation, isolation, dissociation, and isolation system) and comparison elements (non-enzymatic, mechanical, vibration, and sonic). This approach ensured a broad yet focused retrieval of pertinent literature.

Study selection and data collection process

Given the nature of this investigation as a master's thesis, the article selection and data collection processes were undertaken by a single author. This involved screening the identified records based on the predefined eligibility criteria, followed by a thorough examination of the full texts of potentially relevant studies. This approach, while somewhat limited by the capacity of a single researcher, ensured a consistent and focused evaluation of the literature.

Risk of bias across studies

To address the potential risk of bias across the included studies, several measures were implemented. The variability in SVF analysis result variables and the methodological heterogeneity inherent in the investigated isolation techniques necessitated a qualitative synthesis rather than a quantitative meta-analysis. To this end, the Modified IFATS/ISCT Index Score was utilized to provide a comprehensive overview of the outcome measures reported in each study. Additionally, the potential for publication bias, particularly in studies where authors may have conflicts of interest, was carefully considered. Disclosure agreements and funding sources were examined for each study to assess the risk of bias and ensure transparency in the reported findings. The Office of Health Assessment and Translation (OHAT) Risk of Bias Tool for Human and Animal Studies was used to assess the risk of bias and internal validity[18]. Six questions from the tool relating to cross-sectional research were assessed for each study. Each question required a score that reflected the risk of bias: As per the original tool, '++' reflects a low risk of bias, '+' reflects probably a low risk of bias, '-' reflects probably a high risk of bias, and '- -' (double negative) reflects a high risk of bias.

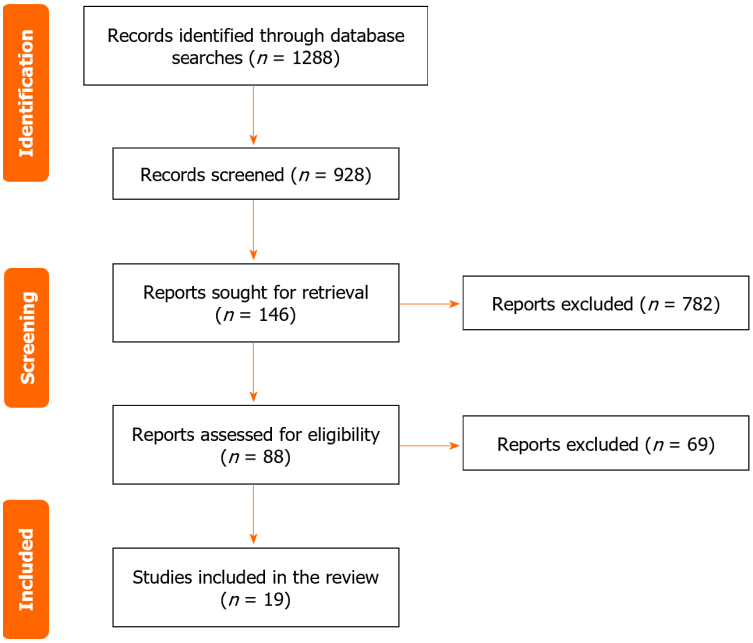

Figure 1 Preferred reporting items for systematic reviews and meta-analyses flow diagram of the included studies.

RESULTS

The outcomes of this systematic review, synthesized in a Prisma flowchart in Figure 1, highlight the rigorous methodology adopted from the initial literature search to the final inclusion of studies. The review was initiated with an exhaustive search across multiple databases, yielding a preliminary tally of 1288 articles. This was supplemented by five additional studies identified from alternative sources, bringing the total to 1293 articles considered for inclusion. The elimination of duplicates pared down this number to 928 unique articles, indicating the extensive nature of the initial search and the importance of reducing redundancy to streamline the review process.

A critical screening of titles further narrowed the pool to 146 articles, with 782 being excluded due to their lack of alignment with the review's stringent preliminary criteria. This step is essential in ensuring that only articles with the most relevant content proceed to the next stage, thereby maintaining the precision and focus of the review. The in-depth evaluation of 88 full-text articles for eligibility resulted in the exclusion of 58 articles. The reasons for these exclusions were varied but primarily related to a divergence from the review's central theme or failure to meet the established inclusion criteria, emphasizing the review's commitment to methodological rigor and thematic relevance. Particular attention was given to the types of studies considered, with a focus on excluding reviews (systematic reviews and literature reviews), surveys, case reports, and other research designs not directly contributing to the review's objectives. This led to the exclusion of 12 studies[11,14,19-28] due to factors such as thematic inconsistency and methodological flaws, highlighting the critical evaluation process in maintaining the integrity of the review. Furthermore, the research designs of two additional studies[29,30] resulted in their exclusion, underscoring the stringent adherence to the review's methodological standards.

Upon meticulous consideration, 19 articles were selected for inclusion, each offering significant insights into the research question through diverse methodological approaches. This selection underscores the necessity of a systematic and objective evaluation to identify studies that significantly contribute to understanding the topic. These studies collectively span a broad range of methodologies, from quantitative analyses to qualitative assessments, reflecting the complexity of the research field and the evolving nature of its investigative methods. Table 1 presents a detailed comparison of cell separation techniques, from mechanical to enzymatic methods, and their impact on cell yield, viability, and efficiency[31-47]. Techniques such as the vibrating shaker and centrifugation, as employed by Raposio *et al*[31], alongside innovative tools like the LipocubeNano and Tulip NanoTransfer Kit by Cohen *et al*[32], are highlighted for their procedural variability and outcomes in terms of cell viability and counts. The pioneering concept of nanofat by Tonnard *et al*[2] and the comparison of mechanical disaggregation *vs* enzymatic dissociation by Sesé *et al*[33] are notable for demonstrating significant differences in cell yields. Additionally, the table reviews procedural innovations, such as the use of the Lipogems system reported by Bianchi *et al*[34], which indicated a higher percentage of mature pericytes and MSCs, showcasing the critical role of methodology in optimizing cell isolation and viability for therapeutic purposes. The inclusion of data on processing techniques by Bright *et al*[35] and a comparison of cell yields across different systems by Gentile *et al*[36] provide essential insights into the efficiency and effectiveness of various separation methods. Table 2 represents the risk of bias in the included studies based on the OHAT criteria. This comprehensive analysis underscores the methodological nuances that influence the advancement of regenerative medicine and cell-based therapies, serving as a pivotal reference in understanding the landscape of SVF and ADSC separation techniques.

Table 1 Summary of the included studies in the systematic review

Ref.	Method of separation	No. of samples	Open/closed	Time taken	Cell counts	Significance
Raposio *et al*[31]	Vibrating shaker at 6000 rpm for 6 min followed by centrifugation at 1600 rpm for 6 min	-	Open	15 min	125000 nucleated cells per cc of lipoaspirate, but only about 5% of these were found to be progenitor cells	A pellet is formed at the end
Cohen *et al* [32]	LipocubeNano decanted for 3 min in a syringe: First, Port 1 is used to pass the fat graft once, resulting in 1 mm parcel sizes. After that, the fat is transferred 10 times back and forth between Ports 2 and 3, smoothing and homogenizing the fat tissue. Finally, to produce the final product, Nanofat, the fat was transferred once from Port 3 to Port 4 *via* a 500-micron single filter. Tulip NanoTransfer Kit. After decantation for 3 min, transfer millifat from Port 1 to 2. Flush the fat between Ports 2 and 3 for 10 times. Collect the final product by transferring fat from Ports 3 to 4 in a single stroke	10 patients	Closed	Not mentioned (Approximately less than 10 min for both methods)	LipocubeNano resulted in relatively high cell counts (2.24×10^6/mL) and cell viability (96.75%), whereas Tulip's NanoTransfer method resulted in a lower cell count of 1.44×10^6/mL and cell viability of 96.75%	-
Tonnard *et al*[2]	Lipoaspirate is washed and rinsed followed by 30 passes done between two 10 mL syringes connected by leur lock and the resultant whitish fluid is filtered over a sterile nylon cloth	67 cases	Open	Not mentioned (Approximately less than 10 min)	1975000 cells per 100 mL of lipoaspirate	Introduced the concept of nanofat
Sesé *et al* [33]	Enzymatic dissociation using GID stromal vascular fraction protocol. Mechanical dissociation using Tulip. NanoTransfer Protocol	20 for enzymatic and 6 for mechanical	Closed	Not available	Enzymatically dissociated stromal vascular fraction resulted in 0.68 million cells/g lipoaspirate, whereas mechanically disaggregated nanofat resulted in 6.63 million cells/g lipoaspirate	-
Bianchi *et al*[34]	Lipogems	-	Closed	Less than 20 min	The significantly higher percentage of mature pericytes and MSCs, and lower number of hematopoietic elements, than enzymatically digested lipoaspirates	-
Bright *et al* [35]	Centrifugation for 2 min at 200 *g* of lipoaspirate. Followed by an ultrasonic cavitation device probe using Hielschler UP200s set at 50% amplitude and cycle of 0.4 for 1 min with the probe lowered and 30 s at the top of the tube. The resultant fluid is subjected to centrifugation for 5 min at 300 *g* with a temperature not rising above 43 degrees and preferably not over 37 degrees	-	Open	-	169 million cells were injected intraartic-ularly for a patient with anterior cruciate tear mentioned but not specified the volume	Described as a patented procedure. Different modific-ations of this technique also have been described based on the indication and site of therapeutic application
Gentile *et al*[36]	Mystem system – washing and filtration. Fastem- Filtration and centrifugation for 10 min at 1700 rpm	10 for Fastem and 10 for Mystem	Closed for both systems	Not available	Cell yield achieved with Mystem is less than that with Fastem and Cytori (enzymatic isolation technique)	Improved contour seen after breast reconstruction with fat grafts enriched with ADSCs from Fastem (equal to Cytori) greater than Mystem
van Dongen *et al*[22]	Centrifuged at 300 rpm for 2.5 min followed by non-enzymatic dissociation performed by pushing the lipoaspirate to and through a fractionator 30 times. The resultant fluid is centrifuged for 2.5 min at 3000 rpm	-	Open	10 min	2.7×10^6/mL	-
Chaput *et al*[37]	Vortexing and centrifuging. Vibrating shaker for 6 min at 3200 rpm followed by	21	Open	22 min	The percentage of ADSCs in SVF	Final pellet for vortexing and

	centrifugation for 6 min at 558 g followed by 100 micrometre sieves followed by centrifugation for 10 min Dissociation by inter-syringe processing 30 passes through leur lock connected syringes passed through 100-micrometre sieve followed by centrifugation for 10 min		Open	11 mins	extracted by vortexing and centrifugation, dissociation by inter-syringe process, and enzymatic isolation techniques are 5.81 ± 1.3, 38.11 ± 5.1, and 21.45 ± 2.52, respectively	centrifuging Final pellet for dissociation by inter-syringe processing
Copcu *et al* [38]	Centrifuged at 500 *g* for 2 min. Adinizing was first performed with a 4000-micron Adinizer; after approximately 25 passes, the cutting process was continued with the next-smaller diameter disk followed by centrifugation for 6 min at 1600 *g*	24 patients	Open	Not mentioned	93% mean viability and cell counts of 28.66 to 88.88 × 10^6 from 100 mL of condensed fat	Volumes ranging from 3-12 mL can be produced depending on the indication
Rose *et al* [39]	Sedimentation for 1 h, centrifugation at 3000 rpm for 3 min or washing with normal saline combined with 3 min of centrifugation at 3000 rpm	24 fat samples	Open	-	The mean cell count per high-powered field of histologically intact adipocytes was 27.1 for specimens processed by sedimentation, 14.2 for centrifuging, and 11.8 for washing	-
Amirkhani *et al*[40]	Dissected for 10 s using a blender mixer followed by sonic cavitation for 2 min at 18 MHz followed by centrifugation for 10 min at 900 *g* followed by suspension with 150 mM ammonium chloride for 5 min and centrifugation for 5 min at 400 g. The pellets are then resuspended in DMEM supplemented with 10% FBS and then seeded into a T25 culture flask. After 24 h, the adherent cells were used for further confirmation tests. The SVFs harvested by both methods were suspended in PBS and then incubated for 30 min at 4 °C with the antibodies conjugated with FITC against CD34, CD44, CD73, CD90, and CD105 biomarkers	-	Open	Less than 30 min	Viable cells 2.6 × 10^5 cells from 1 mL of fat tissue	-
Victor[41]	Ultrasonic cavitation performed using a 200 W generator (SONIC 200) for a range of 10 to 20 min at a frequency of about 20-30 kHz	-	Open	-	From 2 million up to 22 million stromal vascular cells per mL of adipose tissue	Described as a patented procedure
Domenis *et al*[42]	Fastem - automated system performing filtration and centrifugation for 10 min at 1700 rpm	6	Closed	Not available	Only mentioned that cell yield from Fastem was less than Lipokit and less than Cytori	Enriched grafting has greater subcutaneous thickness
Condé-Green *et al* [43]	Centrifugation followed by vortexing for 3 min. Centrifugation followed by RBC lysis	9	Open	Not available	cSVF of 1.2 × 10^4 per mL for the first method and 2.3 × 10^4 per mL for the second method	Mechanical methods have greater cells positive for CD14 than with enzymatic process which is a marker for monocytes and macrophages
Markarian *et al*[44]	The first method involved RBC lysis of lipoaspirate and then centrifugation for 10 min at 600 *g*. The second and third techniques each included an additional initial stage of centrifugation at 800 *g* and 1280 *g* for 15 min, respectively	10	Open	Not available	The cell yield obtained from collagenase was greater than that of mechanical and trypsin. The second and third methods produced viable cells that had not prolif-erated even after 14 d	-
Shah *et al* [45]	Rigorous washing in PBS with handshaking followed by centrifugation for 15 min at 900 *g*	13	Open	1 h for mechanical and almost 3 h for isolation with collagenase	The mechanical method produced 19 times fewer cells compared to the enzymatic extraction technique	-
Condé-Green *et al*	Lipoaspirate is subjected to RBC lysis followed by 15 min of centrifugation at	10	Open	Not available	The highest concen-tration of ADSCs was	-

[46]	900 *g*				found in the pellet found at the bottom after centrifugation	
Baptista *et al*[47]	The procedure followed in the same sequence. RBC lysis, centrifugation for 15 min at 900 g, resuspension in fetal bovine serum plus dimethyl sulfoxide, cryopreservation at –196 degrees centigrade	13	Open	Mechanical processing required less time	Cell yield was less with mechanical compared to enzymatic processing	Adherent cells were positive for CD44, CD90, CD105, and CD34 and negative for CD45 and CD73

MSCs: Mesenchymal stem cells; ADSCs: Adipose-derived stem cells; SVF: Stromal vascular fraction; FBS: Foetal bovine serum; RBC: Red blood cell; cSVF: Cellular stromal vascular fraction; PBS: Phosphate buffered saline.

Table 2 Risk of bias in the included studies based on office of health assessment and translation criteria

Ref.	Did the selection of study participants appropriate?	Did the study account for confounding and modifying variables?	Were the outcome data complete without attrition bias?	Can we be confident in exposure characterization?	Can we be confident in outcome assessment?	Were all measured outcomes reported?
Raposio *et al* [31]	++	+	++	++	++	++
Chaput *et al* [37]	++	++	+	+	++	+
Cohen *et al* [32]	++	+	+	- (NR)	+	+
Copcu *et al* [38]	++	++	++	+	+	++
Tonnard *et al*[2]	++	+	+	+	+	+
Sesé *et al*[33]	++	+	+	+	+	+
Rose *et al* [39]	++	+	++	++	++	++
Bianchi *et al* [34]	++	++	++	++	++	++
van Dongen *et al*[22]	++	+	+	- (NR)	+	+
Amirkhani *et al*[40]	++	++	++	++	++	++
Victor[41]	++	++	++	++	++	++
Bright *et al* [35]	++	-	+	+	+	+
Domenis *et al*[42]	++	++	+	- (NR)	+	+
Gentile *et al* [36]	++	+	+	+	+	+
Condé-Green *et al* [43]	++	++	++	++	++	++
Markarian *et al*.[44]	++	++	+	++	++	++
Shah *et al* [45]	++	++	++	++	++	++
Condé-Green *et al* [46]	++	++	++	- (NR)	+	+
Baptista *et al*	++	+	+	++	++	++

[47]

NR: Not reported; ++: Reflects a low risk of bias; +: Reflects probably a low risk of bias; -: Reflects probably a high risk of bias.

DISCUSSION

In the evolving landscape of regenerative medicine, the utilization of autologous cellular SVF (cSVF) for therapeutic applications represents a significant advancement. This discussion systematically reviews the efficacy, challenges, and clinical implications of mechanical *vs* enzymatic isolation techniques of cSVF, with a focus on their application in osteoarthritis, chronic wounds, bone and cartilage disorders, and Crohn's disease, and as vectors for drug delivery to malignancies[48-51]. The traditional enzymatic digestion method, while effective, faces several limitations including extensive processing time, high costs, and stringent regulatory challenges as outlined by the United States Food and Drug Administration[52,53].

The advent of mechanical cell separation techniques introduces a promising alternative, offering reduced processing time and potentially lower regulatory hurdles. Techniques such as centrifugation, vortexing, and manual shaking have been developed, yet their clinical applicability remains underexplored due to limited published data[31,36,42,45,54]. This gap underscores the necessity for further empirical evidence to validate the reliability and usefulness of these mechanical methods in clinical settings. Mechanical isolation techniques, including innovative automated systems like Fastem, Mystem, and Lipogems, have shown the potential to enhance outcomes in fat grafting procedures. These systems promise a streamlined isolation process within a single device, potentially mitigating risks of contamination and improving volume retention in breast reconstruction surgeries[36,42]. However, the efficiency of these mechanical methods, especially in terms of cell yield and viability, needs thorough evaluation when compared to traditional enzymatic digestion, which is known for its higher cSVF output.

A critical aspect of mechanical separation is its product outcome. Techniques developed by researchers such as Tonnard *et al*[2] and Bianchi *et al*[34] focus on producing a fat-grafting material rich in viable MSCs rather than isolating cSVF as a standalone product. This approach highlights the variability in mechanical isolation outcomes and their implications for clinical practice, emphasizing the need to delineate between methods aimed at enriching fat grafts *vs* those isolating cSVF for broader therapeutic applications. The time efficiency of mechanical methods presents a significant advantage over enzymatic procedures, with some requiring as brief as 30 s for processing[55]. However, the variability in cell yield, survival, and composition of the SVF obtained through mechanical means raises questions about their efficacy and the potential impact on therapeutic outcomes. Furthermore, the effects of mechanical manipulation on cell integrity and the proliferative potential of ADSCs warrant careful consideration, as repetitive processing may compromise cell yield and increase the risk of contamination[42,44,45,56].

The role of ADSCs, characterized by their immunomodulatory, angiogenic, and multipotent properties, is crucial in the context of fat graft maintenance and overall therapeutic efficacy[11,31,45,47,57,58]. The potential adverse effects of mechanical *vs* enzymatic isolation on these cell populations and their functional capabilities remain a pivotal area for further investigation. This exploration is essential to determine whether the differences in cell output and population composition observed with enzymatic methods translate to superior clinical outcomes, justifying their longer processing time and higher associated costs. Considering the therapeutic potential of enriching autologous adipose tissue transfers with ADSCs, the exploration of mechanical processing techniques becomes imperative. These methods offer a promising avenue for enhancing the outcomes of reconstructive and cosmetic procedures by potentially providing a safer and more efficient alternative to enzymatic digestion[25,59,60]. Nonetheless, the challenge of achieving consistent and replicable results due to the heterogeneous nature of mechanically processed SVF highlights the necessity for standardized procedures and rigorous quality control measures.

The primary limitation of this review lies in the novelty of the mechanical isolation techniques and the corresponding scarcity of comprehensive, large-scale comparative studies. The existing literature, characterized by a diversity of methods, small sample sizes, and a lack of randomized control trials, hampers the ability to draw definitive conclusions about the efficacy and safety of mechanical *vs* enzymatic isolation techniques. This variability and methodological heterogeneity limit the strength of the evidence base, underscoring the need for further research. Specifically, well-designed studies comparing mechanical and enzymatic isolation methods are critical to establishing standardized, efficient, and safe practices that can be broadly implemented in clinical settings. The journey toward optimizing cSVF isolation techniques for clinical application is complex and requires a multifaceted approach to research and development. As the field of regenerative medicine continues to evolve, the quest for effective, efficient, and safe methods of cell isolation remains at the forefront of scientific inquiry. The potential of cSVF to revolutionize the treatment of a wide range of conditions is immense, yet realizing this potential hinges on overcoming the current limitations and advancing our understanding of the best practices for cell isolation and application.

CONCLUSION

This systematic review meticulously evaluates the non-enzymatic methods for isolating the SVF and ADSCs from adipose tissue, offering a comprehensive comparison to the traditional enzymatic approaches. The findings underscore the promise of mechanical isolation techniques in addressing the limitations of enzymatic methods, including reducing

processing time, mitigating regulatory hurdles, and potentially enhancing the safety and efficacy of cell-based regenerative therapies. Despite the demonstrated advantages of mechanical methods, such as increased procedural simplicity and the avoidance of enzymatic reagents, this review also highlights the variability in cell yield, viability, and functional integrity of the isolated cells. The current evidence suggests that while non-enzymatic methods hold significant potential for clinical application, their outcomes are varied and require further investigation to optimize cell quality and therapeutic efficacy. The scarcity of large-scale, randomized controlled trials comparing mechanical and enzymatic isolation methods signifies a crucial gap in the literature, emphasizing the need for standardized methodologies and rigorous research to establish evidence-based practices in the field of regenerative medicine. As the field advances, the development and refinement of non-enzymatic isolation techniques will be critical in realizing the full therapeutic potential of SVF and ADSCs, offering promising avenues for enhancing patient outcomes across a broad spectrum of medical conditions.

FOOTNOTES

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Country of origin: India

ORCID number: Naveen Jeyaraman 0000-0002-4362-3326; Sathish Muthu 0000-0002-7143-4354; Swaminathan Ramasubramanian 0000-0001-8845-8427; Madhan Jeyaraman 0000-0002-9045-9493.

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META-ANALYSIS

Association between tobacco exposure and bladder cancer recurrence: A systematic review and meta-analysis

Lei Xiang, Qi-Qi Xie, Si-Si Xu, Wen-Jie Ruan, Dong-Hui Xu, Yao-Yao Gan, Jia Zuo, Wen-Jun Xu, Zhi-Peng Li

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Lei Xiang, Department of Rehabilitation Medicine, First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Henan Province, China

Qi-Qi Xie, Si-Si Xu, Wen-Jie Ruan, Dong-Hui Xu, Yao-Yao Gan, Jia Zuo, Wen-Jun Xu, Zhi-Peng Li, School of Medicine, Taizhou University, Taizhou 318000, Zhejiang Province, China

Corresponding author: Zhi-Peng Li, MD, Senior Editor, Surgeon, Teacher, School of medicine, Taizhou University, No. 1139 Shifu Avenue, Jiaojiang District, Taizhou 318000, Zhejiang Province, China. lzpzhonghong@126.com

Abstract

BACKGROUND

However, the connection between smoking and the prognosis of patients with bladder cancer remains unclear.

AIM

To determine whether smoking is linked to the recurrence and progression of bladder cancer.

METHODS

As of July 20, 2022, relevant English-language research was identified by searching PubMed, the Web of Science, and the Cochrane Library. We pooled the available data from the included studies using a random effects model. Subgroup analysis and sensitivity analysis were also conducted.

RESULTS

A total of 12 studies were included in this meta-analysis. The combined analysis revealed that tobacco exposure was associated with a significantly greater recurrence rate than nonsmoking status [odd ratios (OR) = 1.76, 95%CI: 1.84-2.93], and the progression of bladder cancer was significantly greater in smokers than in nonsmokers (OR = 1.21, 95%CI: 1.02-1.44). Stratified analysis further revealed that current smokers were more likely to experience relapse than never-smokers were (OR = 1.85, 95%CI: 1.11-3.07). Former smokers also had a greater risk of relapse than did never-smokers (OR = 1.73, 95%CI: 1.09-2.73). Subgroup analysis indicated that non-Caucasians may be more susceptible to bladder cancer recurrence than Caucasians are (OR = 2.13, 95%CI: 1.74-2.61).

CONCLUSION

This meta-analysis revealed that tobacco exposure may be a significant risk factor

for both the recurrence and progression of bladder cancer.

Key Words: Smoking; Bladder; Cancer; Recurrence; Progress

Core Tip: In this meta-analysis, 12 studies were included to investigate the connection between smoking and the prognosis of bladder cancer patients. The results showed that tobacco exposure was associated with a significantly greater recurrence rate and faster progression of bladder cancer than nonsmoking status. Subgroup analysis further revealed that current and former smokers had a greater risk of relapse than did never smokers, and non-Caucasians may be more susceptible to bladder cancer recurrence than Caucasians are. Therefore, smoking is a major risk factor for bladder cancer recurrence and progression, and cessation of smoking is recommended. Regular follow-up and treatment are crucial for reducing the risk of smoking.

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INTRODUCTION

Bladder cancer is a prevalent urological malignancy worldwide. It affects a significant number of individuals and remains a common cancer type, particularly in developed countries[1]. Bladder cancer leads to hundreds of thousands of deaths annually[2]. The incidence of bladder cancer is affected by sex-related factors. The occurrence of this disease is approximately three to four times greater in males than in females. However, women with bladder cancer often receive a diagnosis at a later stage, at which point the disease tends to be more severe and associated with a poorer prognosis[3]. The 5-year recurrence rate for bladder cancer varies depending on several factors, such as tumor grade, the number of primary tumors, the prostate-specific antigen test score, and the tumor-to-lymph node metastasis classification. Low-risk patients have a 5-year recurrence rate of approximately 57%, while intermediate- and high-risk patients have recurrence rates of approximately 67% and 77%, respectively[4,5]. Due to its high recurrence rate, bladder cancer necessitates substantial medical resources for detection and management each year. The management of these conditions places a significant burden on healthcare systems globally.

Tobacco smoking remains a significant global public health concern and leads to more than 5 million deaths each year [6]. In China alone, 2 million people lose their lives with smoking-related illnesses[7]. Smoking has been firmly established as a critical risk factor for numerous diseases, including chronic respiratory conditions such as chronic obstructive pulmonary disease and cardiovascular disease[8]. Additionally, smoking is intricately linked to various forms of cancer and is a particularly significant risk factor for some cancers[9]. Notably, smoking is the most significant predictor of bladder cancer development, and the risk of this disease has increased over time. Prior research has shown that smoking accounts for a population attributable risk of 50%-65% in men and 20%-30% in women, and smoking triples the risk of bladder cancer compared to never smoking[10]. While several cohort studies have examined the relationship between smoking and bladder cancer prognosis-including survival rates and tumor recurrence the findings remain inconclusive. For instance, an epidemiological study by Hagiwara *et al*[11] reported that a positive smoking history and male sex were independent risk factors for bladder tumor recurrence after radical nephroureterectomy. Higher smoking levels were associated with a greater likelihood of upper urothelial carcinoma incidence of bladder tumor recurrence, with shorter years of smoking and nonsmoking patients exhibiting lower rates of tumor recurrence than long-term smokers[12]. Other research groups have reported similar positive correlations between smoking and increased relapse rates[13]. In contrast, some retrospective cohort studies have also shown that smoking is not an independent risk factor for recurrence[14-16]. Given these mixed results, we conducted a meta-analysis aiming to synthesize the available evidence on the relationship between smoking and both bladder cancer recurrence and progression.

The study was carried out in accordance with the PRISMA[17] statement.

MATERIALS AND METHODS

Ethical approval

Given that this meta-analysis is based on previously published data and does not involve any individual-level data collection or analysis, ethical approval is not needed for this study. This study adheres to the ethical guidelines and best practices for meta-analyses, ensuring a rigorous and objective synthesis of the available evidence.

Search strategy

We conducted a comprehensive literature search of Medline (PubMed), Web of Science, and the Cochrane Library covering the period from the establishment of each database to July 2022. Our search strategy included a combination of keywords and medical subject headings terms related to smoking, urinary bladder neoplasms, malignant tumor of the urinary bladder, cancer, tumor, bladder, cancer recurrence, and various combinations of these terms. We also manually checked the reference lists of all identified studies and related reviews to ensure comprehensive coverage. Additionally, we searched the Clinical Trials website (clinicaltrial.gov) for relevant unpublished studies as of July 20, 2022. The flowchart in Figure 1 summarizes the identification and evaluation process of the studies included in this review.

Inclusion and exclusion criteria

Two independent reviewers (Li ZP and Xie QQ) evaluated the eligibility of each study using standardized criteria. The inclusion criteria were as follows: (1) Original research article; (2) had undergone bladder cancer surgery; (3) smoked long-term compared to never or former smokers or were currently smoking as an indicator of exposure; (4) had risk estimates [hazard ratios (HR), risk ratios (RR), odd ratios (OR)] with corresponding 95%CI for the study results or detailed baseline and follow-up data in the authors' report to be able to calculate the above indicators; and (5) papers are written and published in English. To minimize errors and biases in the pooled data, a unified standard for the definition of smoking was established. Any active exposure to tobacco was defined as "tobacco exposure". Patients who were still smoking at the time of bladder cancer diagnosis or who stopped smoking within one year of diagnosis were defined as "current smokers". "Former smoker" was defined as a patient who had quit smoking at least one year before bladder cancer diagnosis. Never smoking from birth to diagnosis of bladder cancer was defined as "never" or "never". The bladder cancer outcome definitions analyzed in this meta-analysis included "disease recurrence" and "disease progression".

After analysis by researchers, articles were screened according to the following criteria to exclude the following: (1) Did not report available data, such as conference abstracts, expert opinions, comments, or letters to the editor; (2) were well-published articles or articles with data reuse; (3) had flawed research designs and low quality assessment scores; (4) had incorrect statistical methods that could not be corrected, could not be provided or could not be transformed into ORs (RR, HR) or their 95%CI, or measured data that did not provide means and standard deviations; and (5) had smoking exposure categories and corresponding effect values that were not clearly described. If two studies reported the same or overlapping populations, only the study with the largest sample size was included; if the studies included different and related exposures or stratified analyses, both studies were included.

Data extraction

Two researchers (Si-Si Xu and Wen-Jie Ruan) independently extracted information from the eligible studies using standardized data collection forms. The extracted information included the first author, year of publication, study design, database and country, follow-up time, sample size, outcome, age, sex, time of diagnosis, surgical method, interventions, and smoking status.

Quality assessment

The quality of the included studies was assessed using the Newcastle-Ottawa Quality Assessment Scale[18] for cohort studies. This scale rates the quality of selection, comparability, and outcome quality of the included articles. After independent scoring by two reviewers, the studies included in this study were identified as follows: 1 high-quality study, 10 medium-quality studies, and 1 low-quality study.

Risk of bias analysis

The RevMan (Review Manger 5.4.1) for assessing risk of bias was utilized to conduct an analysis of bias risk.

Statistical analysis

The combined analysis revealed that most studies classified smoking status as smoking, quitting smoking, or never smoking. To unify the standards and eliminate the influence of subtle differences in the definitions of smoking among different articles, we used current smoking, former smoking and never smoking to define smoking status.

Statistical heterogeneity was assessed using the Cochrane chi-square test (Q test) ($P < 0.1$ was considered to indicate significant statistical heterogeneity). HR and 95%CI were calculated using Cox regression for statistical analysis. Study-specific risk estimates were pooled by random or fixed effects meta-analyses. The association between smoking status and postoperative bladder cancer outcomes was assessed in detail using forest plots.

Subgroup analysis and meta-regression analysis were also conducted based on surgical method (radical cystectomy *vs* transurethral resection of the bladder), disease stage (muscle invasive bladder cancer, nonmuscle invasive bladder cancer and bladder cancer), geographic region (United States, Europe or Asia), and study design (single center and the center) to identify potential sources of heterogeneity. Sensitivity analyses were performed by the back-off method (removing one item at a) to test whether the results were influenced by a particular study.

Furthermore, a funnel plot assessment was applied to capture potential publication bias and to examine the impact of publication bias on the validity of the estimates. Statistical analyses were conducted using SPSS 28.0 (IBM, Chicago, IL, United States) and RevMan software, version 5 (http://ims.cochrane.org/revman). $P < 0.05$ was considered to indicate statistical significance unless the article specifically stated otherwise.

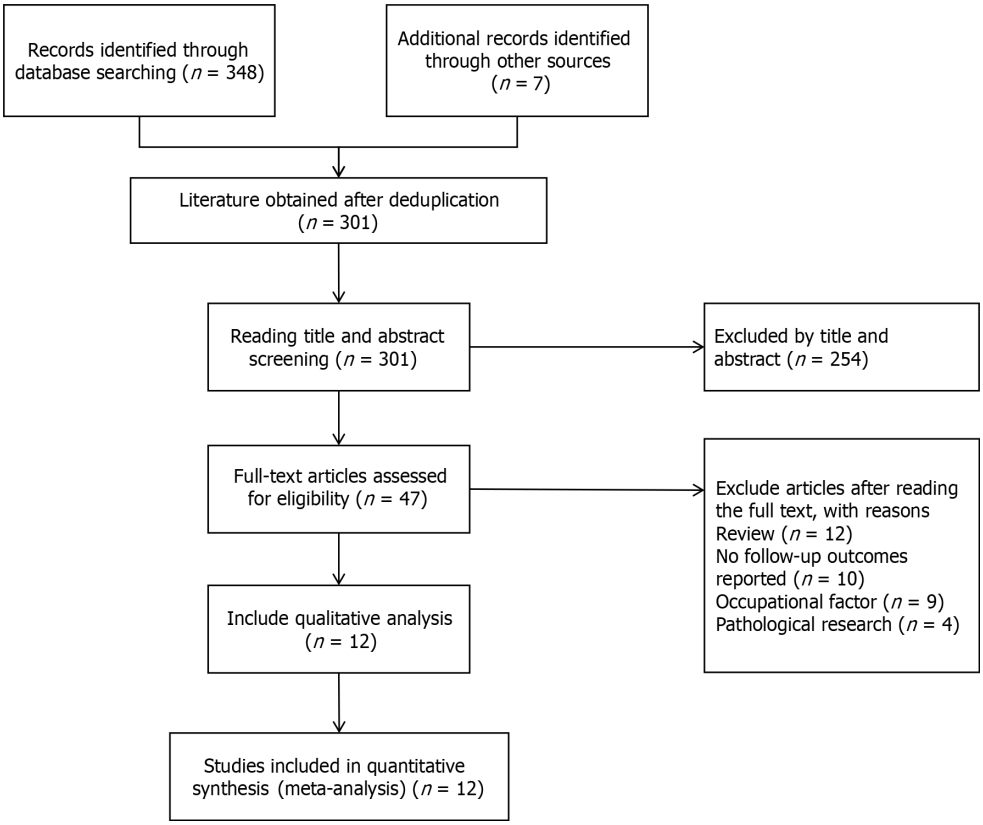

Figure 1 Flow diagram of study selection.

RESULTS

Study characteristics

This passage is a summary of a meta-analysis that aimed to assess the impact of smoking status on bladder cancer outcomes. The researchers conducted a comprehensive literature search and identified 186 articles from PubMed, 123 articles from the Web of Science, 32 articles from the Cochrane Library, and 7 articles manually. After screening and evaluation by reviewers, 12 studies were included in the meta-analysis[11,12,14,16,19-26]. The included studies were conducted in different regions, including Europe (5 studies)[16,21-24], Asia (5 studies)[11,12,19,25,26], and North America (2 studies)[14,20]. A total of 5817 patients were included in the meta-analysis, and most of those studies provided detailed follow-up data on tobacco exposure. Yuruk *et al*[26] study recruited 187 participants and grouped them according to their smoking status[16]. On the other hand, Grotenhuis *et al*[16] included a total of 1459 patients from 1995 to 2006 after three expansions. However, only 66% of the patients responded, resulting in a cohort of 963 patients for the study. These figures demonstrate the extensive variation in participant demographics across various studies, emphasizing the significance of conducting research on diverse patient populations to achieve a more comprehensive understanding of the impact of smoking on bladder cancer outcomes.

The findings from the meta-analysis suggest that smoking status is linked to both the recurrence and progression of bladder cancer. These results from this study can offer valuable insights to healthcare professionals and patients about the impact of smoking on bladder cancer outcomes, thus informing future research in this area.

Qualitative assessment

The Newcastle-Ottawa Quality Assessment Scale was used to evaluate the quality of each study, with scores ranging from 6 to 9 (with a mean of 7.42), indicating a generally acceptable methodological approach. Table 1 lists the scores for each individual study, while Table 2 provides a detailed breakdown of the scoring criteria.

Tobacco exposure and bladder cancer recurrence

Twelve articles were included in the analysis, with only 11 articles considered for the nonsmoking comparison, as shown in Figure 2. The meta-analysis revealed a significant association between tobacco exposure and an increased risk of bladder cancer recurrence in patients who smoked compared to those who had never smoked, with an OR of 1.84 (95%CI: 1.15-2.93, Figure 2A). Notably, substantial heterogeneity was observed across the included studies [I-squared statistic (I^2) = 91%, Q = 111.48, P < 0.00001 for heterogeneity]. Furthermore, current smoking status was also associated with an elevated risk of bladder cancer recurrence compared to never smoking status, with an OR of 1.85 (95%CI: 1.11-3.07, Figure 2B). Again, significant heterogeneity was observed across studies (I^2 = 91%, Q = 105.66, P < 0.00001 for heterogeneity). Additionally, previous smoking was found to be associated with an increased risk of bladder cancer recurrence

Table 1 Characteristics of the included studies

Ref.	Area	Sort	Period	Mean follow-up time (months)	Sample size	Disease stage	NOS score	Cur:For:Non
Michalek *et al*[14], 1985	United States	Retrospective	1963-1975	N/A	354	NMIBC	7	132:128:94
Grotenhuis *et al*[16], 2015	Netherlands	Forward-looking	2007-2012	12	963	NMIBC	8	292:490:181
Wyszynski *et al*[25], 2014	Lebanon	Forward-looking	1994-2002	37	857	NMIBC	8	214:379:123
Chen *et al*[19], 2007	Taiwan	Retrospective	1997-2005	N/A	206	NMIBC	6	78:64:64
Leibovici *et al*[20], 2005	United States	Forward-looking	1995-2003	15	519	NMIBC	7	185:239:95
Yuruk *et al*[26], 2017	Turkey	Forward-looking	2013-2014	32	187	NMIBC	9	114:35:38
van Osch *et al*[24], 2018	United Kingdom	Forward-looking	2005-2011	51	722	NMIBC	8	336:283:103
Ogihara *et al*[12], 2015	Japan	Retrospective	1995-2012	N/A	634	NMIBC	8	181:154:299
Rava *et al*[21], 2018	Spain	Forward-looking	1998-2001	N/A	936	MIBC	7	401:369:166
Hagiwara *et al*[11], 2013	Japan	Retrospective	1994-2010	N/A	245	NMIBC	7	72:52:121
Serretta *et al*[22], 2013	Italy	Retrospective	2002-2003	48	395	NMIBC	7	127:171:97
Serretta *et al*[23], 2020	Italy	Forward-looking	2008-2012	N/A	194	NMIBC	7	67:127

NMIBC: Non-muscle- invasive bladder cancer; MIBC: Muscle-invasive bladder cancer; NOS score: Newcastle-Ottawa scale.

compared to never smoking, with an OR of 1.73 (95%CI: 1.09-2.73, Figure 2C), albeit with substantial heterogeneity across studies (I^2 = 88%, Q = 86.26, P < 0.00001 for heterogeneity). However, when comparing previous smoking status to current smoking status, no significant association was observed with bladder cancer recurrence, yielding an OR of 1.01 (95%CI: 0.74-1.38, Figure 2D). Nonetheless, substantial heterogeneity was still evident across studies (I^2 = 83%, Q = 64.02, P < 0.00001 for heterogeneity).

Tobacco exposure and bladder cancer progression

Twelve articles were included in the analysis, as shown in Figure 3. The meta-analysis revealed that tobacco exposure was associated with an increased risk of bladder cancer progression compared with never smoking status, with an OR of 1.21 (95%CI: 1.02-1.44, Figure 3A). No significant heterogeneity was observed across studies (I^2 = 0%, Q = 1.46, P = 0.69). Current smoking status was not associated with an increased risk of bladder cancer progression compared with never smoking status, with an OR of 1.24 (95%CI: 0.99-1.56, Figure 3B). No significant heterogeneity was observed across studies (I^2 = 29%, Q = 4.21, P = 0.24). Previous smoking status was not associated with an increased risk of bladder cancer progression compared with never smoking status, with an OR of 1.15 (95%CI: 0.96-1.38, Figure 3C). No significant heterogeneity was observed across studies (I^2 = 0%, Q = 2.10, P = 0.55). However, when comparing previous smoking status to current smoking status, no significant association was observed with bladder cancer progression, yielding an OR of 1.17 (95%CI: 0.96-1.43, Figure 3D). Substantial heterogeneity was observed across studies (I^2 = 76%, Q = 12.60, P = 0.006). In summary, the meta-analysis suggested that tobacco exposure is associated with an increased risk of bladder cancer progression compared to never smoking. However, the results for current and previous smoking statuses were inconclusive due to insufficient data and significant heterogeneity across studies. Additional research is needed to further explore the impact of smoking on bladder cancer progression and to address the limitations of the current analysis.

Subgroup analysis and sensitivity analysis

Subgroup and meta-regression analyses were also conducted to explore heterogeneity among studies examining the association between current smoking status and disease recurrence (Table 3). Notably, significant heterogeneity was observed. However, we performed a sensitivity analysis to assess the impact of individual studies on the pooled results by excluding each study in turn. The results indicated that the significant association between current smoking status and disease recurrence was consistent and robust (data not shown). Therefore, despite the observed heterogeneity, the conclusion that current smoking status is associated with an increased risk of disease recurrence is supported by the

Table 2 Assessment of quality of studies by the Newcastle–Ottawa scale

Ref.	Selection			Comparability			Outcome			Score
	1	2	3	4	5a	5b	6	7	8	
Michalek *et al*[14], 1985	1	1	1	1	1		1	1		7
Grotenhuis *et al*[16], 2015	1	1	1	1	1	1	1	1		8
Wyszynski *et al*[25], 2014	1	1	1	1	1		1	1	1	8
Chen *et al*[19], 2007	1		1	1	1	1	1			6
Leibovici *et al*[20], 2005	1	1	1	1			1	1	1	7
Yuruk *et al*[26], 2017	1	1	1	1	1	1	1	1	1	9
van Osch *et al*[24], 2018	1	1	1		1	1	1	1	1	8
Ogihara *et al*[12], 2015	1	1		1	1	1	1	1	1	8
Rava *et al*[21], 2018	1		1	1	1	1	1	1		7
Hagiwara *et al*[11], 2013	1		1		1	1	1	1	1	7
Serretta *et al*[22], 2013	1		1	1	1		1	1	1	7
Serretta *et al*[23], 2020	1	1	1	1	1	1			1	7

1 = the exposed cohort is representative; 2 = Study cohort with unexposed; 3 = Exposure factor identified; 4 = No positive results at the start of the study; 5 = Cohort comparability based on design or analysis [(a) Study age controls; (b) Study controls for any additional factors); 6 = Outcome assessment is reliable; 7 = Follow-up time is long enough; 8 = Adequacy of cohort follow-up.

sensitivity analysis. Additional research is needed to further explore the impact of smoking on disease recurrence and to address the limitations of the current analysis.

Publication bias assessment

By creating and analyzing funnel plots, it was observed that there was potential publication bias between tobacco exposure and bladder cancer recurrence (Figure 4A), which may have affected the reliability of the pooled results. However, no obvious publication bias was observed between tobacco exposure and bladder cancer progression (Figure 4B), indicating that the pooled results are robust. These findings suggest that additional studies are needed to confirm the association between smoking and bladder cancer recurrence, particularly to address potential publication bias and other sources of heterogeneity. We used the RevMan bias risk tool to carry out risk assessment, and the results showed that all the articles included in this study had a low risk (Figure 5). The findings revealed that no significant high risk of bias was identified across any of the included studies. Notably, three studies exhibited unclear risk of bias with regard to other potential biases, while one study demonstrated unclear risk of bias, specifically in terms of selection bias.

DISCUSSION

Bladder cancer is a common malignancy worldwide, and there are significant sex differences in its incidence[27,28]. Tobacco smoking is a well-established risk factor for bladder cancer, as exposure to tobacco carcinogens increases the morbidity and mortality associated with this disease[29]. However, most of those previous studies focused on prevention and clinical treatment, and there is limited research on the prognosis of bladder cancer, particularly the impact of tobacco smoking on patients after surgical treatment[30]. Currently, there is no conclusive evidence that tobacco smoking increases the risk of bladder cancer recurrence or progression after surgery. Therefore, further research is needed to clarify the relationship between tobacco exposure and bladder cancer prognosis, including the impact of smoking cessation on disease outcomes.

To our knowledge, this is the first systematic epidemiological assessment of the association between smoking status and bladder cancer patient outcomes in the past five years. A meta-analysis of 12 cohort studies with a total of 5817 bladder cancer patients was conducted to provide stable and reliable results. The outcomes of surgical treatment for bladder cancer include disease recurrence, disease progression, and cancer-specific mortality. The pooled results suggest that tobacco exposure may increase the risk of bladder cancer recurrence after surgery. Both current smoking and previous smoking were found to be independent risk factors for bladder cancer recurrence, but there was no significant difference between current smoking and previous smoking in terms of bladder cancer recurrence after surgery (OR = 1.01, 95%CI: 0.74-1.38). Additionally, we found that tobacco exposure was significantly associated with bladder cancer progression, but when patients were stratified, the associations of current smoking status and previous smoking status

Table 3 Summary of meta-analysis results on smoking status and disease recurrence in patients with bladder cancer

Analysis specification	*n*	Tobacco exposure *vs* never smoking		Current smoker *vs* never smoker		Former smoker *vs* never smoker		Current smoker *vs* former smoker	
		OR (95%CI)	I^2 (%)	OR (95%CI)	I^2 (%)	OR (95%CI)	I^2 (%)	OR (95%CI)	I^2 (%)
All	12	1.84 (1.15-2.93)	91	1.85 (1.11-3.07)	91	1.73 (1.09-2.73)	88	1.01 (0.74-1.38)	83
Race			91		90		88		83
Caucasian	7	1.33 (1.12-1.58)		1.32 (1.09-1.59)		1.29 (1.07-1.56)		1.03 (0.89-1.18)	
Noncaucasian	5	2.13 (1.74-2.61)		2.24 (1.78-2.83)		1.76 (1.39-2.23)		1.16 (0.93-1.45)	
Study design			91		91		88		83
Multi	6	1.98 (1.68-2.32)		2.07 (1.73-2.48)		1.67 (1.39-2.01)		1.21 (1.04-1.40)	
Single	5	1.09 (0.87-1.37)		1.00 (0.78-1.30)		1.12 (0.87-1.43)		0.81 (0.65-1.00)	
Sort			91				88		84
Retrospective	5	3.23 (2.59-4.02)		3.23 (2.52-4.14)		3.15 (2.44-4.08)		1.04 (0.83-1.32)	
Forward	6	1.05 (0.89-1.24)		1.09 (0.91-1.32)		0.97 (0.81-1.17)		1.10 (0.95-1.28)	
Disease stage			91		91		88		83
NMBIC	10	1.93 (1.65-2.26)		1.83 (1.54-2.18)		1.81 (1.52-2.15)		0.94 (0.81-1.09)	
Others	2	1.00 (0.78-1.29)		1.20 (0.91-1.59)		0.85 (0.65-1.12)		1.38 (1.11-1.72)	
Research cycle			91		91		88		83
End of before 2010	5	1.26 (1.01-1.56)		1.22 (0.95-1.56)		1.25 (0.99-1.59)		0.93 (0.76-1.14)	
End after 2010	7	1.88 (1.59-2.21)		1.92 (1.60-2.31)		1.59 (1.32-1.92)		1.14 (0.98-1.33)	

OR: Odd ratios; I^2: I-squared statistic; NMBIC: Non-muscle- invasive bladder cancer.

with postoperative bladder cancer progression were not statistically significant. These findings suggest that smoking cessation may be beneficial for improving the prognosis of bladder cancer patients. However, further research is needed to confirm these results and to explore the impact of smoking cessation on bladder cancer outcomes.

The pooled results from the meta-analysis showed that current and former smokers had a significantly greater risk of experiencing disease relapse than patients who had never smoked. Additionally, bladder cancer progression was significantly faster in individuals exposed to tobacco. However, the differences in postoperative recurrence between patients who were current smokers and those who were previously smoking were not well characterized, suggesting that the relationship between tobacco exposure and bladder cancer recurrence may not be dose dependent. Furthermore, tobacco exposure was significantly associated with cancer progression, but the roles of current and previous smoking in cancer progression have not been well validated. The mechanisms underlying the association between tobacco exposure and bladder cancer recurrence and progression are thought to be complex and multifactorial. Strong carcinogens in tobacco, such as coal tar, polycyclic aromatic hydrocarbons, aromatic amines, and nitrosamines[31,32], are absorbed into the bloodstream through inhalation and transported to the kidneys, where they are concentrated in the urine. This process results in exposure of the bladder epithelium to carcinogens, leading to cellular damage and an increased risk of bladder cancer development. Additionally, long-term exposure to smoking carcinogens may lead to cumulative molecular alterations that adversely affect bladder cancer biology and clinical behavior, promoting growth and motility. Furthermore, continuing smoking may weaken the immune response to bladder cancer, leading to an increased risk of recurrence and death. These findings suggest that smoking cessation may be beneficial for improving the prognosis of bladder cancer patients. However, further research[32] is needed to confirm these results and to explore the impact of smoking cessation on bladder cancer outcomes.

These findings suggest that tobacco exposure may have a longer-term effect on bladder cancer recurrence, even after smoking cessation. This highlights the importance of smoking cessation for bladder cancer patients to improve their prognosis. However, the exact mechanism by which smoking cessation reduces subsequent tumor recurrence in patients with bladder cancer remains to be elucidated. Recent studies have shown that nicotine, a major component of tobacco smoke, activates multiple signaling pathways through nicotinic acetylcholine receptors. The MAPK/ERK, PI3K/Akt, and JAK/STAT pathways are associated with tumorigenesis, tumor progression, and acquisition of treatment resistance. These findings suggest that the effects of tobacco exposure on bladder cancer recurrence may be complex and multifactorial and involve various signaling pathways and mechanisms. Therefore, further research is needed to explore the detailed mechanisms by which smoking cessation reduces subsequent tumor recurrence in bladder cancer patients. This may lead to the development of effective strategies for improving the prognosis of bladder cancer patients who smoke or have a history of smoking. Ogihara *et al*[12] suggested that nicotine exposure in tobacco may induce tumor cell proliferation by activating the PI3K/Akt/mTOR pathway both *in vitro* and *in vivo*. This activation of signaling pathways

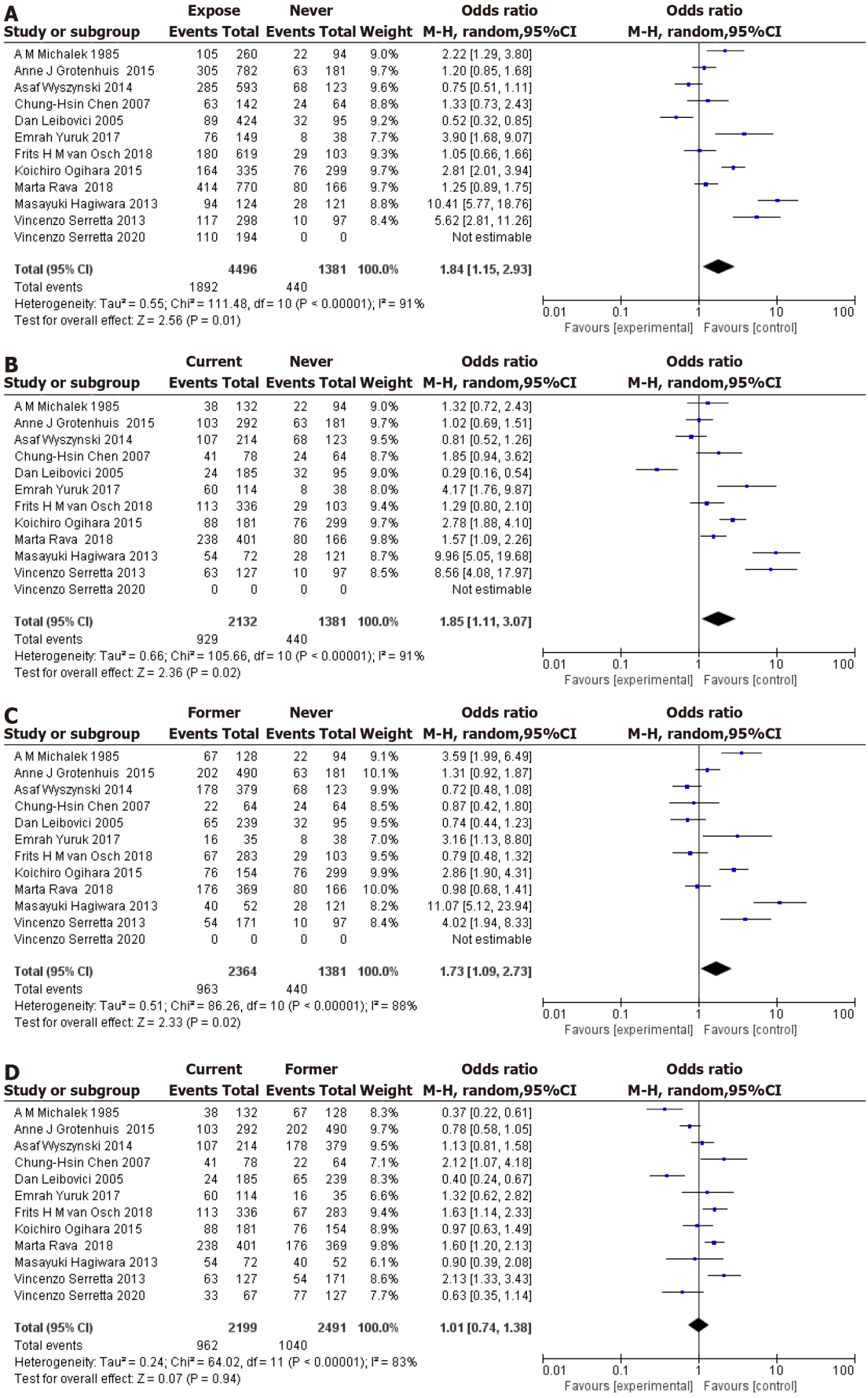

Figure 2 Meta-analysis of studies on the associations between the risk of bladder cancer recurrence and current smoking, previous smoking, and tobacco exposure. A: Tobacco exposure *vs* recurrence in never smokers; B: Current smoking *vs* recurrence in never smokers; C: Former smoking *vs* recurrence in never smokers; D: Current smoking *vs* recurrence in former smokers.

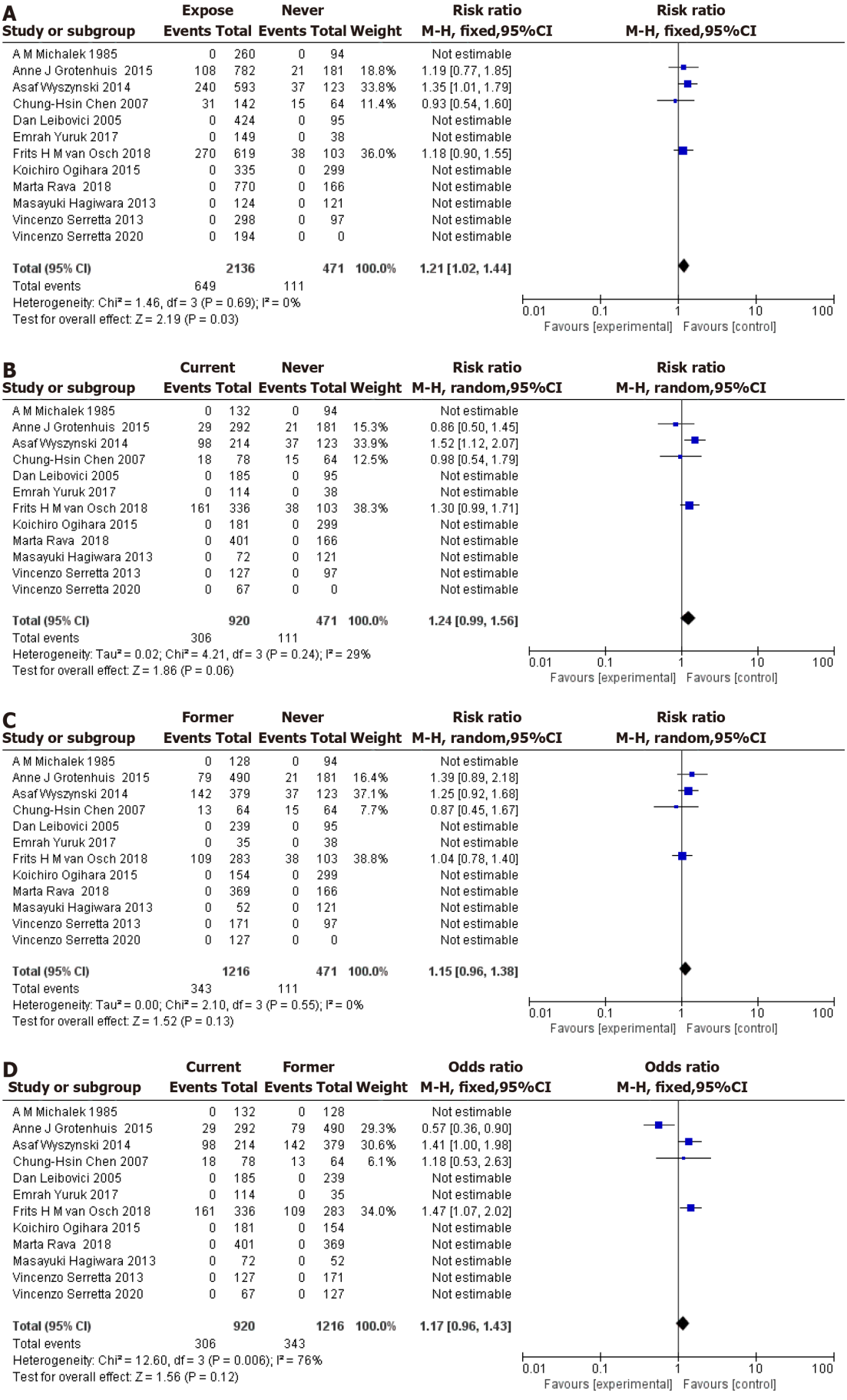

Figure 3 Meta-analysis of studies on the associations between the risk of bladder cancer progression and current smoking, previous

smoking, and tobacco exposure. A: Tobacco exposure *vs* progression; B: Current smoking *vs* progression in never smokers; C: Former smoking *vs* progression in never smokers; D: Current smoking *vs* progression in former smokers.

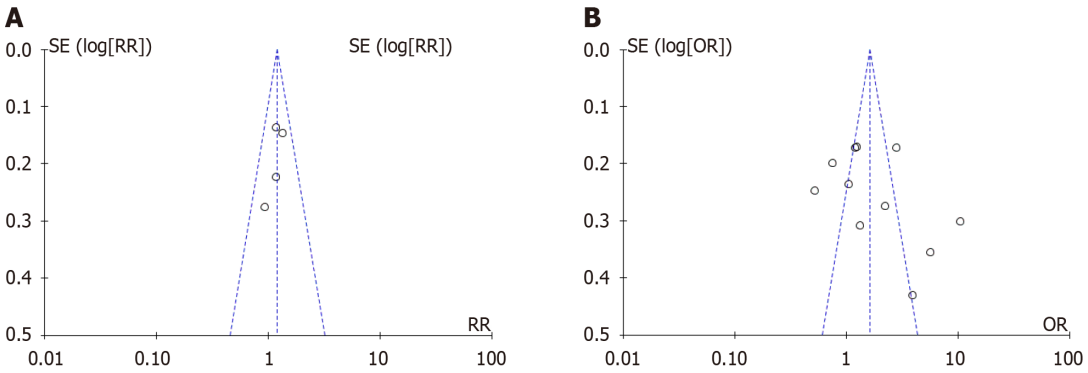

Figure 4 Tobacco exposure-recurrence and tobacco exposure-progression funnel plots. A: Tobacco exposure and relapse; B: Tobacco exposure and progress.

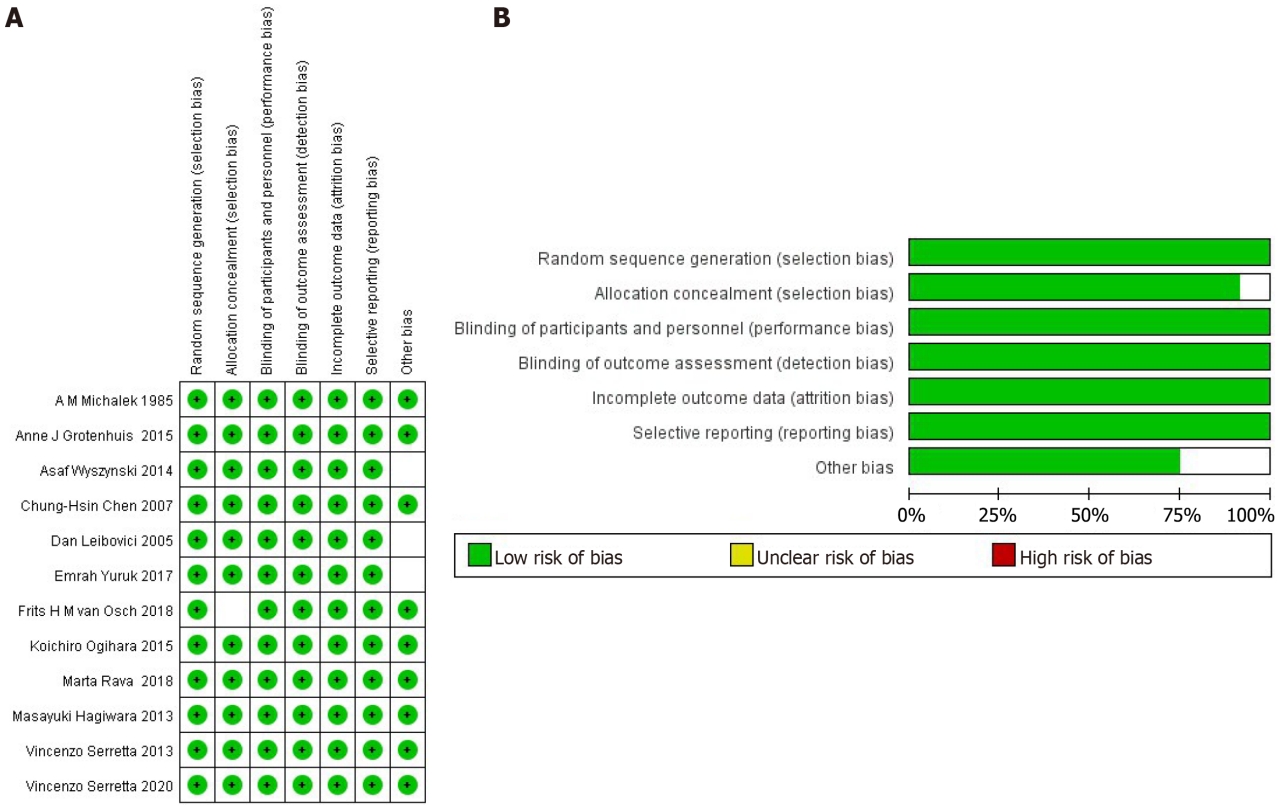

Figure 5 Assessment of bias risk in included studies. A: Risk of bias summary, review the judgments about each risk of bias item for each included study; B: Risk of bias graph, the judgments about each risk of bias item are presented as percentages across all included studies.

by nicotine can lead to irreversible harmful cell activation, indicating that smoking cessation may not completely prevent the progression and recurrence of bladder cancer. The metabolic cycle of nicotine in the body is relatively short, lasting only 2-6 h[33], but the activation of these cancer-promoting signaling pathways by nicotine can have long-term effects on tumor growth and recurrence. Therefore, smoking cessation is still an important measure for reducing the risk of bladder cancer recurrence, but smoking cessation may not completely eliminate this risk. Future research should focus on exploring the detailed mechanisms by which smoking cessation reduces subsequent tumor recurrence in patients with bladder cancer. This may lead to the development of effective strategies for improving the prognosis of bladder cancer patients who smoke or have a history of smoking. The limitations of this study include the retrospective nature of some of the included studies, which may introduce recall bias. Additionally, there was significant heterogeneity in the studies regarding tobacco exposure, including different types of tobacco products, exposure modalities, and surgical techniques.

This heterogeneity may have influenced the overall results, leading to a potential futility association between tobacco exposure and bladder cancer outcomes. It should also be noted that the effects of current and previous smoking on bladder cancer progression were not statistically significant, possibly due to the limited number of studies addressing this topic. The lack of adjustment for potential confounders in some studies may have also affected the results. In conclusion, the results of this meta-analysis suggest that tobacco exposure is significantly associated with postoperative recurrence and progression of bladder cancer. However, larger epidemiological studies with longer follow-up periods are needed to confirm these findings and further explore the mechanisms underlying the effects of tobacco exposure on bladder cancer outcomes.

CONCLUSION

This meta-analysis suggested that tobacco exposure may increase the risk of bladder cancer recurrence and progression after surgery. However, there was no significant association between current or previous smoking and postoperative cancer progression. Smoking is a major risk factor for bladder cancer, and cessation of smoking is recommended. Notably, quitting smoking may not completely eliminate this risk. Regular follow-up and treatment are crucial for reducing the risk of bladder cancer recurrence and progression in smokers. Additionally, genetic, environmental, and lifestyle factors may also influence bladder cancer risk, and smokers should consider these factors when taking preventive measures.

FOOTNOTES

Author contributions: Xiang L wrote the original draft; Xie QQ performed the methodology, software and data organization; Xu SS and Ruan WJ performed the data curation; Xu DH, Gan YY, Zuo J and Xu WJ formally analyzed and wrote the original manuscript; Li ZP for conceptualization, writing, reviewing and editing. All the authors participated in drafting the manuscript, and all the authors read, contributed to, and approved the final version of the manuscript.

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ORCID number: Lei Xiang 0000-0002-0784-0980; Qi-Qi Xie 0000-0002-0723-3996; Sisi Xu 0009-0001-3737-612X; Wen-Jie Ruan 0009-0007-3036-3936; Dong-Hui Xu 0000-0002-4365-9257; Yao-Yao Gan 0009-0009-0250-7095; Jia Zuo 0000-0001-8044-1161; Wen Jun Xu 0009-0001-8635-6141; Zhi-Peng Li 0000-0002-0355-7889.

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CASE REPORT

Patellar reconstruction in primary total knee arthroplasty using bone chips from routine cuts: A case report and review of literature

Juan Ignacio Perez-Abdala, Franco L De Cicco, Tomas Nicolino, Juan Astoul

Specialty type: Orthopedics

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's scientific quality classification

Grade A (Excellent): 0
Grade B (Very good): B
Grade C (Good): C
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Juan Ignacio Perez-Abdala, Franco L De Cicco, Tomas Nicolino, Juan Astoul, Institute of Orthopaedics "Carlos E. Ottolenghi", Italian Hospital of Buenos Aires, Capital Federal 1198, Argentina

Corresponding author: Juan Ignacio Perez-Abdala, MD, Surgeon, Orthopaedic Department, Italiano Hospital of Buenos Aires, Peron 4190, Capital Federal 1199, Argentina.
ignacio.perez@hospitalitaliano.org.ar

Abstract

BACKGROUND

Total patellectomy is currently reserved for exceptional cases, such as recalcitrant patellofemoral instability and comminuted fractures, due to its demonstrated negative impact on knee biomechanics. Therefore, managing patellectomy is crucial to mitigate its inherent deleterious effects. Various techniques have been described, including autologous or allogeneic bone grafts for reconstruction and soft tissue realignment to enhance the extensor mechanism.

CASE SUMMARY

A 73-year-old male underwent a patellectomy due to a comminuted fracture, subsequently developing osteoarthritis and experiencing a decline in functional status. Concurrent with total knee replacement, we conducted a patellar reconstruction, incorporating routine bone cuts and utilizing bone chips to fashion a new patella. This intervention resulted in the restoration of full extension and improvement of knee function.

CONCLUSION

Patellar reconstruction demonstrates benefits on knee mechanics and stabilization, contributing to enhanced outcomes and satisfaction following knee replacement. We present an affordable technique for managing patellectomized patients undergoing total knee replacement.

Key Words: Patellectomy; Patella; Reconstruction; Knee arthroplasty; Autologous bone; Knee osteoarthritis; Case report

Core Tip: Patients who have undergone patellectomy tend to experience worse outcomes following total knee arthroplasty than those with an intact patella. We present a 73-year-old male who, after a left patellectomy due to a comminuted fracture, developed osteoarthritis leading to detriment knee function. We reconstructed the patella using bone chips from routine bone cuts during the total knee arthroplasty. Post-surgery, the patient reported improved knee function and satisfaction. The key takeaway is that patellar reconstruction enhances knee function mainly due to its mechanical advantages, leading to better outcomes. The technique performed is safe and feasible.

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INTRODUCTION

Total patellectomy was a prevalent procedure in past decades, primarily employed to address conditions such as chondromalacia, patellofemoral arthritis, patellofemoral dislocation, and comminuted fractures[1-5]. In contemporary times, an enhanced understanding of the patella's role in biomechanical knee function, coupled with advancements in surgical techniques, has relegated this radical surgery to a salvage option[6]. Nonetheless, patients with patellectomy sequelae persist or when there is no alternative to avoid patellectomy, as is the case with malignant tumors. Concerning the mechanical properties of the patella, numerous studies have demonstrated that its absence can lead to a decrease in tibial torque force by up to 30%, resulting in a reduction of the lever arm between the quadriceps tendon and the center of rotation, causing an extension deficit[1,7,8]. Additionally, the femorotibial reaction force amplifies the tangential force, potentially contributing to osteoarthritis development[9]. Other adverse effects include quadriceps muscle wasting, loss of joint protection, an unfavorable aesthetic appearance, and instability of the remaining tendon, which may lead to degeneration and, ultimately, rupture[2,10-13]. Decades ago, the theory of the four-bar linkage system was introduced, depicting the patella as a stabilizer in the sagittal plane, limiting anterior femoral displacement during knee flexion. However, contemporary perspectives cast doubt on the full acceptance of this role of the patella[10-15].

The literature supports that patients without patella requiring total knee arthroplasty (TKA) report poorer overall outcomes compared to those with an intact patella, encompassing factors such as range of motion, knee function, patient satisfaction, and complication rates[14-20]. Given these considerations, surgeons must evaluate how to reconstruct the patella, considering the need to preserve biomechanical properties. Patellar reconstruction might be performed using various methods, including autologous bone grafts, allogeneic extensor or bone grafts, and, less commonly, metal augmentations[6,10,21-29]. Additionally, alternative techniques involving soft tissue have been described[30]. However, the existing literature is characterized by scarce evidence and heterogeneous samples, preventing the establishment of reliable recommendations regarding the optimal approach. In this context, our goal is to introduce an alternative technique for patellar reconstruction in patellectomized patients while simultaneously performing a TKA.

CASE PRESENTATION

Chief complaints

A 73-year-old male patient presented at the hospital with severe knee pain and difficulty ambulating.

History of present illness

The patient underwent a total patellectomy of his left knee for a comminuted fracture sixty years ago, which led to the development of osteoarthritis with valgus deformity. The patient required a walker to ambulate and experienced high levels of pain, rated at 9/10 on the visual analogue scale (VAS) in activity, which resulted in daily opioid consumption.

History of past illness

The patient underwent a total patellectomy of his left knee for a comminuted fracture sixty years ago. Moreover, he was developing pain, subjective knee instability, and lesser strength than another knee.

Personal and family history

He denies any personal or family disease that could be significant for the pathology and outcomes.

Physical examination

On physical examination, the patient had 10° of deficit active extension and flexion greater than 120°. Despite coronal misalignment, as shown in Figure 1, the medial collateral ligament was competent in varus-valgus stress examination with a firm endpoint. The Knee Society Score (KSS) was 29 points for the knee and 45 points for function, and the strength was 3/5 according to the Medical Research Council (MRC) scale[31].

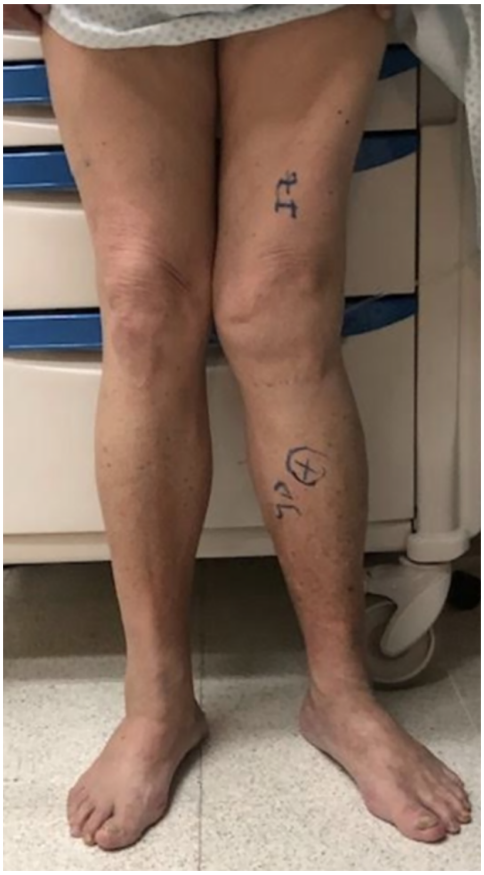

Figure 1 Pre-operative physical examination. It shows valgus misalignment in the left knee on the coronal view.

Laboratory examinations

His laboratory testing was within normal parameters.

Imaging examinations

X-rays showed severe femorotibial osteoarthritis, valgus misalignment, absence of the patella, and areas of tendon calcification (Figure 2). Following a thorough discussion of potential treatment options with the patient, we proposed a TKA with patellar reconstruction, to which the patient willingly consented.

FINAL DIAGNOSIS

Left knee osteoarthritis with sequelae of patellectomy.

TREATMENT

We performed a standard medial para-patellar approach, exposing the femur and tibia and making cuts using intra and extra-medullary jigs, respectively. Subsequently, achieving balance in all ranges of motion and successfully restoring the mechanical axis. A cemented semi-constrained implant with a long tibial stem was then used.

Proceeding to the patellar reconstruction, we morselized the remaining bone from the tibia and femur cuttings with a rongeur, obtaining cortico-cancellous chips. To determine the accurate positioning and size of the neo-patella, we measured the dimensions of the contralateral patella beforehand, trying to replicate the Insall-Salvati ratio[32]. Notably, the patellar tendon was hypertrophy, measuring approximately 2 cm in thickness. Addressing this, we made a 5 cm longitudinal incision on the medial aspect of the tendon, creating an intra-tendon pocket while leaving a 7 cm remnant of the patellar tendon (Figure 3). The amount of bone chips used varied; however, the objective was to reconstruct a neo-patella that would reposition the extensor apparatus away from the knee's center of rotation, mirroring a healthy knee. The pocket was filled with bone chips using a press-fit technique. Before closing the tendon pocket, we conducted tests to ensure correct patellar tracking and to verify the absence of excessive pressure or instability in the extensor mechanism. Finally, we closed the pocket using strong non-absorbable sutures, specifically Ti-Cron™ #0 (Figure 4). Immediate postoperative x-rays, primarily in the lateral view, revealed the neo-patella positioned slightly high, measuring 5 cm in

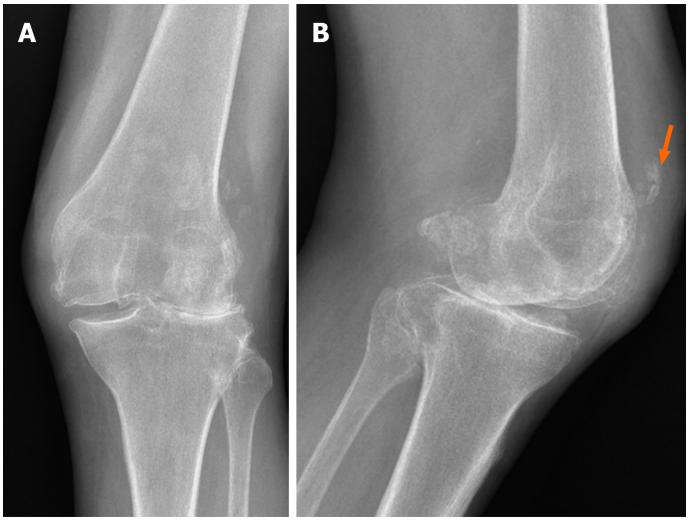

Figure 2 Pre-operative X-rays. A and B: It shows severe valgus osteoarthritis in the anteroposterior view (A) and the absence of the patella with areas of tendon calcification (orange arrow) in the lateral view (B).

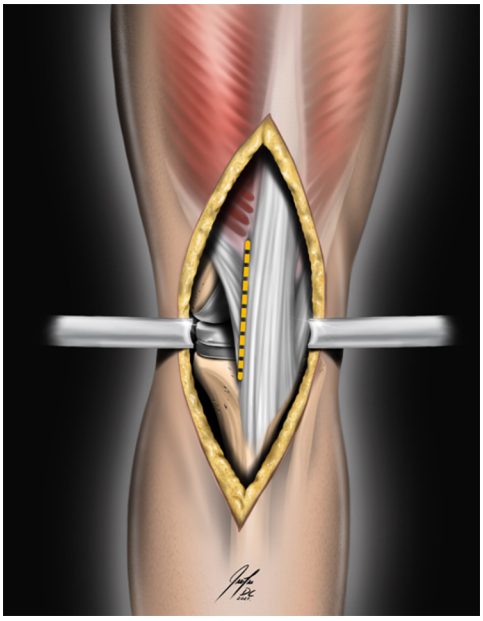

Figure 3 The picture illustrates the localization of the tendon incision to create an intra-tendon pocket (dashed line).

length and 1.5 cm in thickness (Figure 5).

OUTCOME AND FOLLOW-UP

The standardized rehabilitation protocol was initiated on the first day postoperative, incorporating passive and active mobility exercises, quadriceps isometric exercises, and progressive weight-bearing using a walker, as tolerated by the patient. He used a walker for the initial fifteen days, then a cane until definitively discontinuing walker support at the two-month postoperative mark, achieving a stable and pain-free gait. By five months postoperative, he reached full active extension (0° to 115°) and 5/5 muscle strength according to the MRC scale. At the last follow-up, two years after the surgery, he preserved full extension and quadriceps strength (Figure 6). The KSS improved to 68 points for knee and 80 points for function, and the VAS decreased to 2/10. Also, the patient reported complete satisfaction with the procedure. Upon the two-year follow-up, radiographs demonstrated an improved shape of the neo-patella, with no signs of bone resorption. The lateral image revealed a persistent increased Insall-Salvati ratio of 1.6, although the patient denied any symptoms related to patellar stability (Figure 7).

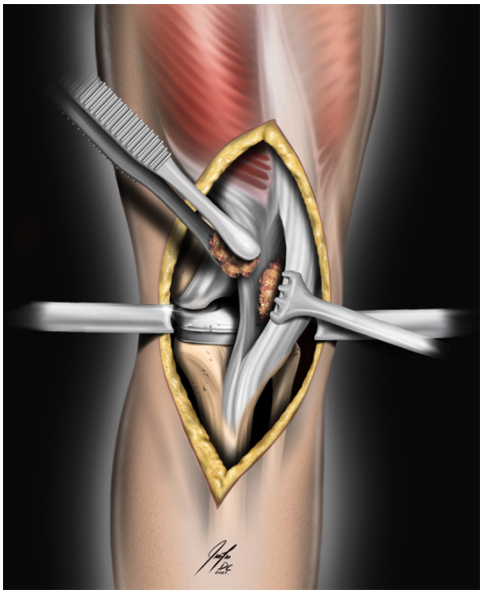

Figure 4 The picture demonstrates the fitting of autogenous bone chips from the remaining routine cuts into the intra-tendon pocket.

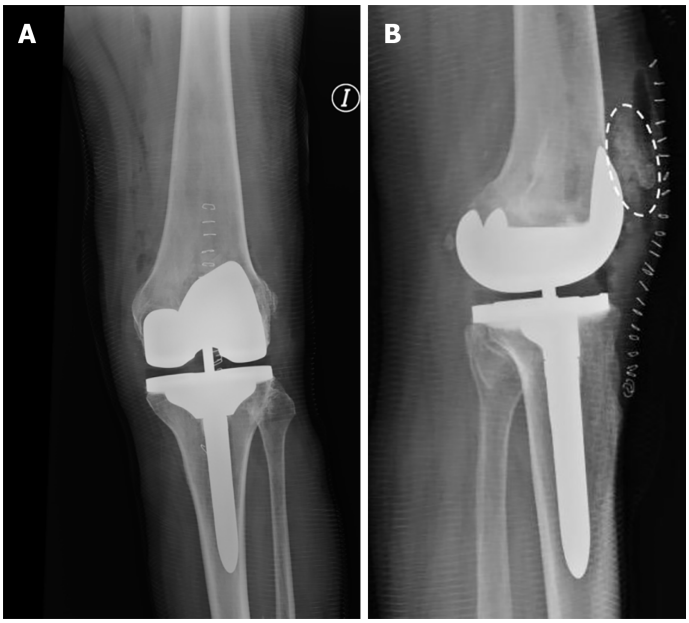

Figure 5 Immediate postoperative X-rays. A: It demonstrates good alignment of total knee arthroplasty in the anteroposterior view; B: In the lateral view, the neo-patella evidence is filled with bone chips (dashed circle), giving the appearance of a fragmented patella.

DISCUSSION

We are presenting a case involving patellar reconstruction in a patient with a history of patellectomy who developed knee osteoarthritis and experienced impaired knee function. Although the specific contribution of a missing patella to the development of osteoarthritis may be a subject of debate, it is well-documented that its absence can substantially affect the function of the extensor mechanism[7-13]. Following the simultaneous performance of total knee arthroplasty and patellar reconstruction, the patient exhibited enhanced knee function, particularly emphasizing the restoration of the extensor mechanism[1,2,11,33].

Several studies have consistently demonstrated that, in patellectomized patients, the clinical and functional outcomes after TKA are lower than those in patients with an intact patella[14,16-19]. In a study by Haque *et al*[20] two groups were followed for an average of 9.5 years: one comprised patellectomized patients, and the other had patients with an intact patella. Most cases were managed with TKA, utilizing either posterior-stabilized (PS) or cruciate-retaining (CR) designs based on the surgeon's discretion[20]. The study demonstrated lower survivorship free from revision in the group without a patella (82%) compared to the group with an intact patella (94%). Additionally, the patellectomized group had a higher rate of complications, mainly attributed to infections and instability. The increased instability in the patellec-

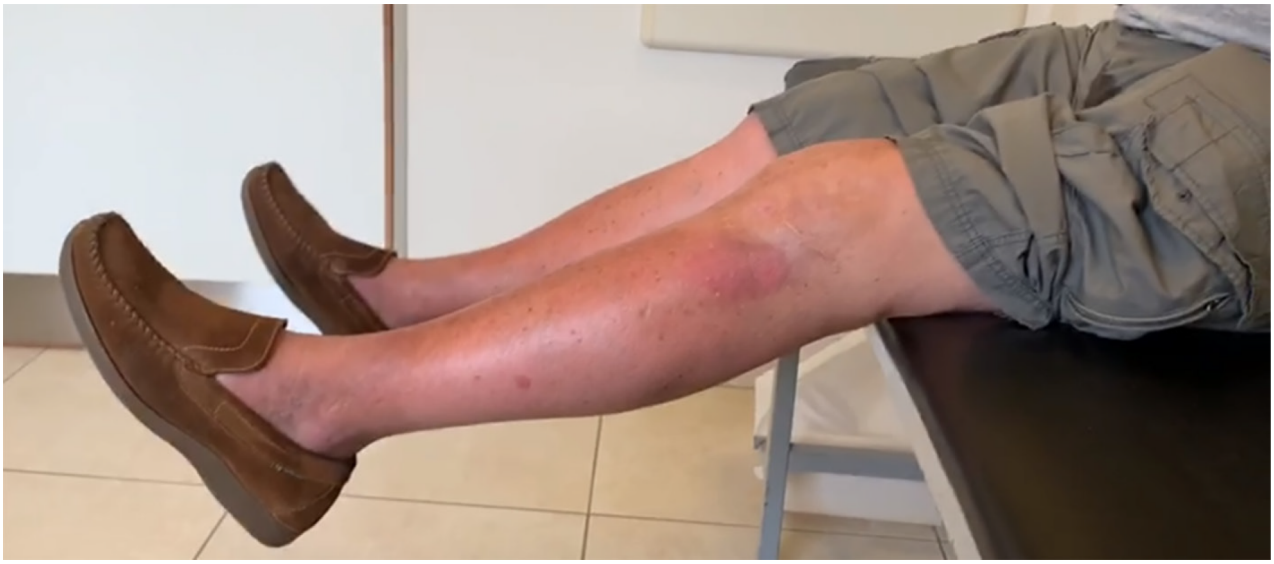

Figure 6 Post-operative clinical image. It was taken at five months showing complete recovery of knee extension.

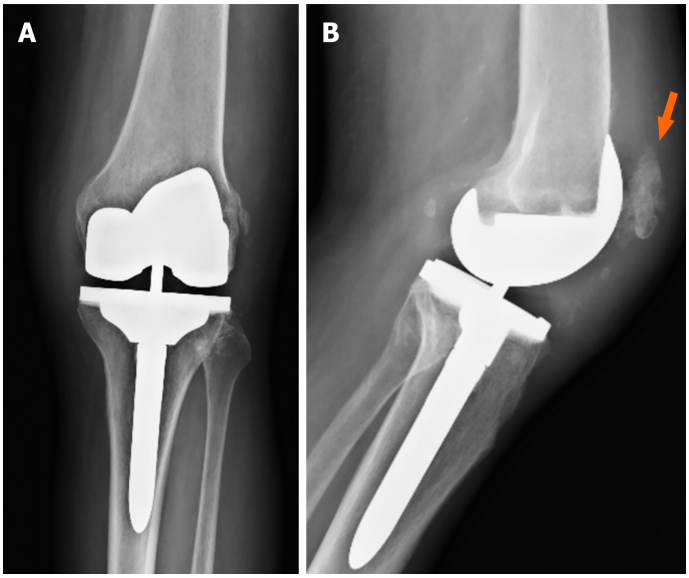

Figure 7 Two-year follow-up. A: No signs of prosthesis loosening are apparent in either the anteroposterior or lateral views; B: Moreover, in the lateral view, there is no evidence of neo-patellar resorption; instead, it shows an improved shape closely resembling the native patella. It is important to note that the patella appears slightly higher, although this does not manifest as clinical instability.

tomized group was attributed to quadriceps weakness due to the absence of the patella. The concept of four-bar linkage system can explain this fact, where the patella could serve as a stabilizer in the sagittal plane, limiting anterior femoral displacement during knee flexion. Moreover, the absence of patellar bone anteriorly, coupled with soft tissue laxity or instability, may lead to significant soft tissue effusions. In turn, this can contribute to challenges in wound healing and an increased risk of infection[20]. Considering these outcomes, the authors suggest patellar reconstruction procedures as a viable alternative for patients with a history of patellectomy.

An area of debate for individuals who have undergone patellectomy revolves around the choice of implant. We opt for a semi-constrained design due to the perceived intra-operative stability, evident in both sagittal and coronal planes, surpassing that of the PS design. Some authors, like Paletta *et al*[15], advocate for PS designs, reporting improved functional and clinical scores with this choice[15,34]. These findings align with the earlier-discussed four-bar linkage system. However, Haque *et al*[20] observed similar complication and revision risks with both CR and PS designs, acknowledging a potential bias in design selection, particularly in cases with preoperative quadriceps weakness and consistent instability where PS was preferred for added sagittal stability. Other reports, such as the meta-analysis by Asadollahi *et al*[19], have indicated similar outcomes between these designs[17-19]. Nonetheless, the latter study suggests that PS designs may provide enhanced sagittal stability for patellectomized patients lacking a competent posterior cruciate ligament. Additionally, unicompartmental replacements, though limited to case reports, have shown favorable short-term functional and clinical scores[35,36]. However, we assert that the choice of implant design should primarily be

Table 1 Patellar reconstruction techniques

Ref.	Type of patellar reconstruction	Number of patients	Follow-up	Outcomes	Complications
Buechel[26], 1991	Bone auto and allograft - iliac crest, femoral resection and bone bank	6 (7 knees)	24 to 125 months	Full ROM, strengthening, pain free gait and no signs of graft resorption	1 CRPS
Kulkarni *et al*[28], 1999	Extensor mechanism allograft	1	3 yr	Improve ROM, strength, level of pain and no signs of graft resorption	None
Tirveilliot *et al*[25], 2003	Bone auto and allograft - 6 tibial plateau / 1 fragment of femoral head	7	1.5 months to 6 yr	IKS pre/postoperative; Knee: 41/78; Function: 35/72	4 were removed for migration
Lakshmanan and Wilson[10], 2004	Bone autograft - entire tibial plateau	1	9 months	KSS postoperative; Knee: 90; Function: 92	None
Busfield and Ries [27], 2006	Extensor mechanism allograft	7 (9 knees)	39 to 48 months	KSS pre/postoperative; Knee: 59/85; Function: 63/67	2 infections 1 patellar resorption
Kwong and Desai [29], 2008	Prosthetic augment	7	6 to 21 months	Initial recovery was good, complications emerged over time	3 loosening 2 continuing pain
Pang and Sathappan[23], 2008	Bone autograft - distal medial femoral condyle	1	2 yr	Full ROM, strengthening, pain free gait and no signs of graft resorption	None
Jabbar and Ruiz [22], 2009	Bone autograft - posterior lateral femoral condyle	1	4 yr	Full ROM, strengthening, pain free gait and no signs of graft resorption	None
Daentzer *et al*[24], 2012	Bone autograft - iliac crest	13	3 to 92 months	KSS postoperative; Knee 67.3; Function 57.5	3 infections (one removed patella); 1 patellar instability; 1 fracture of ASIS[e]
George *et al*[21], 2017	Bone autograft - medial femoral condyle	2	1.5 to 4 yr	Full ROM, strengthening, pain free gait and no signs of graft resorption	None
Giessler and Hendrich[37], 2016	Hybrid - vascularized bone graft with metal augmentation	1	6 months	Full ROM, strengthening, pain free gait and no signs of graft resorption	None
Gómez-Palomo *et al*[6], 2019	Extensor mechanism allograft	1	5 yr	Full ROM, strengthening and pain free gait	None

ROM: Range of motion; CRPS: Complex regional pain syndrome; IKS: International Knee Society; KSS: Knee Society Score; ASIS: Anterior superior iliac spine.

guided by stability testing in both the preoperative and intraoperative phases, with particular attention to cases involving patellar reconstruction, as it can contribute to increased stability. Several techniques for patellar reconstruction have been described, encompassing autograft bone, allograft extensor mechanisms, prosthetic augments, and soft tissue reconstruction (Table 1).

Autograft bone can be harvested from the medial femoral condyle or tibial plateau through distal cutting[21,23-25]. While both techniques are generally reliable, our case presented challenges due to the size due to the size of the medial femoral condyle, particularly in cases of severe valgus knee osteoarthritis. It's worth noting that reinforcing sutures is crucial to prevent neo-patella migration, as emphasized by the authors. Alternatively, the iliac crest bone can be utilized, yielding satisfactory results in select patients[24,26]. However, it is being acknowledged that morbidity at the harvest site and complications are not uncommon[24]. Additionally, immediate aggressive rehabilitation is strongly recommended for achieving optimal outcomes[23-26].

Allogeneic extensor grafts remain an irreplaceable option in patients with tumor pathology[6]. Nonetheless, there is a growing concern regarding the heightened risk of infection, prompting surgeons to approach this technique with extreme care[27,28]. More recently, tantalum augmentation has been proposed as an alternative for these patients, yet its inferior outcomes and high revision rates dissuade its widespread adoption[29]. Moreover, a patellar reconstruction employing a hybrid technique has been described, integrating prosthetic augmentation with a vascularized bone graft harvested from the scapula, resulting in successful outcomes[37]. Soft tissue reconstruction emerges as a viable option, aiming to restore patellar function, with most reconstructions focusing on advancing the vastus medialis oblique[12,30].

While the precise contribution of patellar reconstruction to enhanced knee function remains elusive, the literature cited indirectly supports our favorable clinical and functional outcomes following its implementation. We attribute our success to the meticulous placement of the fragmented autograft bone in a press-fit manner within the intra-tendon pocket, facilitating optimal patellar tracking, and securing closure with strong sutures to prevent graft migration. Despite the

potentially compromised vascularity of the extensor mechanism due to prior surgeries, our patellar reconstructions have maintained bone integrity on radiographs two years post-operation, showing no signs of resorption[21,37]. Though the long-term viability of the bone graft remains uncertain, our hypothesis asserts that maintaining the occupied space through patella reconstruction, whether by original bone or fibrous tissue replacement, may suffice to preserve the mechanical integrity of the patella and yield satisfactory outcomes. Additionally, rigorous postoperative rehabilitation under the guidance of an experienced physiotherapist is imperative. This technique offers several advantages, including a low complication rate, cost-effectiveness, technical reproducibility, and adherence to biomechanical principles. However, limitations may arise from the thin and poor-quality tendon of the extensor mechanism, posing a rupture risk if a pocket is attempted. Therefore, despite encouraging outcomes observed in just one case, further research is imperative to ascertain the efficacy and safety of this technique in a larger prospective cohort.

CONCLUSION

A patellar reconstruction is a viable option for patellectomized patients with clinical impairment. It restores the knee biomechanics and enhances outcomes for patients undergoing primary or revision total knee arthroplasty TKA. Our technique is novel and reproducible without adding morbidity and cost to surgery; therefore, it would be a therapeutic alternative for patellectomized patients.

FOOTNOTES

Author contributions: Perez Abdala JI contributed to the conception and design of the study, acquisition of data, and drafting the article; De Cicco FL contributed to creating illustrations and critically analyzing the data; Astoul J and Nicolino T contributed to the conception and design of the study; all authors have read and approved the reviewed manuscript.

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Country/Territory of origin: Argentina

ORCID number: Juan Ignacio Perez-Abdala 0000-0002-5398-6876; Franco L De Cicco 0000-0001-9844-140X; Tomas Nicolino 0000-0002-9550-3713; Juan Astoul 0000-0001-6798-8242.

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LETTER TO THE EDITOR

Japanese candlestick charts for diabetes

Diana Boj-Carceller

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Diana Boj-Carceller, Department of Endocrinology and Nutrition, Miguel Servet University Hospital, Zaragoza 50009, Spain

Corresponding author: Diana Boj-Carceller, MD, PhD, Doctor, Teaching Assistant, Department of Endocrinology and Nutrition, Miguel Servet University Hospital, Paseo Isabel La Católica, 1-3, Zaragoza 50009, Spain. dbojcarc@gmail.com

Abstract

Continuous glucose monitoring (CGM) is a popular technology among the diabetic population, especially in patients with type 1 diabetes and those with type 2 diabetes treated with insulin. The American Diabetes Association recommends standardization of CGM reports with visual cues, such as the ambulatory glucose profile. Nevertheless, interpreting this report requires training and time for CGM to be cost-efficient. In this work it has been proposed to incorporate Japanese candlestick charts in glucose monitoring. These graphs are used in price analysis in financial markets and are easier to view. Each candle provides extra information to make prudent decisions since it reports the opening, maximum, minimum and closing glucose levels of the chosen time frame, usually the daily one. The Japanese candlestick chart is an interesting tool to be considered in glucose control. This graphic representation allows identification of glucose trends easily through the colors of the candles and maximum and minimum glucose values.

Key Words: Japanese candlestick chart; Candlestick chart; Ambulatory glucose profile; Glucose monitoring; Continuous glucose monitoring; Hypoglycemia

Core Tip: This original brief report proposes and explains how to use a graph from financial markets to synthesize the continuous glucose monitoring (CMG) data. Specifically, Japanese candlestick chart for diabetes would save time for both the doctor and the patient in detecting glycemic trends and maximums and minimums at a glance. This work responds to the need to standardize and simplify CMG reports in order to improve decision making in diabetes control.

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TO THE EDITOR

The present work arises from the observed similarity between price action in financial markets and glucose fluctuations in patients with diabetes. In recent years, continuous glucose monitoring (CGM) has become popular due to the accessibility of flash glucose-sensing technology. Diabetic patients can read their interstitial glucose minute by minute without the use of annoying needles. CGM also alerts users to trends. These systems have been greatly received[1,2].

The 2023 Standards of Medical Care in Diabetes[3] recommend standardization of CGM reports with visual indications, such as ambulatory glucose profile (AGP), with a level of evidence 'E' based on expert consensus or clinical experience. This can help both the patient and specialist to have a better interpretation of the data to guide treatment decisions.

The AGP is a summary of glucose values for the reporting period, with the median (50%) and other percentiles displayed as if they occurred on a single day. Time in range is associated with a risk of microvascular complications. In 2017, the following glucose targets were established by consensus: 70-180 mg/dL for most people with diabetes and 63-140 mg/dL for pregnant women with diabetes[4]. Time below-range and above-range are useful for evaluation of the therapeutic plan.

Interpretation of an AGP report is not obvious, and some rules in the form of the algorithm are necessary for the doctor and patient to understand and improve glycemic control[5]. Although the AGP report provides a useful overview of the glycemic profile, it is also necessary to review daily glucose profiles to ensure that important glycemic excursions are not missed (*e.g.*, an individual with a severe hypoglycemic or hyperglycemic event that may not be revealed in the AGP report)[6].

The limitations of needing to train the doctor and patient in order to interpret a summary and needing to carefully scrutinize the daily record imply an intellectual and time-consuming effort. This fact may justify the inconsistency of randomized controlled trials made with flash glucose-sensing technology in terms of improving glycemic control and preventing severe hypoglycemia in both type 1 and type 2 diabetes, with studies showing positive[7,8] and negative results[2,9]. In observational and real-life studies, individuals with type 1 diabetes generally improve glycemic control with these devices but not in all cases[10]. It seems that the doctor and patient have an excess of information that is not always profitable. For this technology to be cost efficient we must be able to extract the maximum relevant information in the shortest time possible[5].

Daily glucose profiles are line graphs that have glucose (mg/dL) on the ordinate axis and time on the abscissa from midnight to midnight. Price in financial markets has traditionally been represented in the West in a similar way: as a line graph that shows a line joining the closing prices for each moment in time[11]. In this way, a similarity between glucose behavior in the daily glucose profile and the price in financial markets can be seen.

Japanese candlestick charts, also known as candlestick charts, are easier to view than line charts, and they provide extra information. The Japanese have been using them for centuries. Nison[12] introduced them to the Western world a few years ago, but it was Homma, a rice merchant, who developed them in the 18th century. He managed to identify that the price of rice was influenced not only by supply and demand but also by traders' emotions. As their name indicates, Japanese candlestick charts are a graphic representation in the form of candles of price action in financial markets. They represent price movement but include more information within each candle. Additionally, Japanese candlestick patterns show and predict price variations. Traders prefer to read candlestick charts because they provide much more information than a line chart and can be much more useful in making prudent decisions[13].

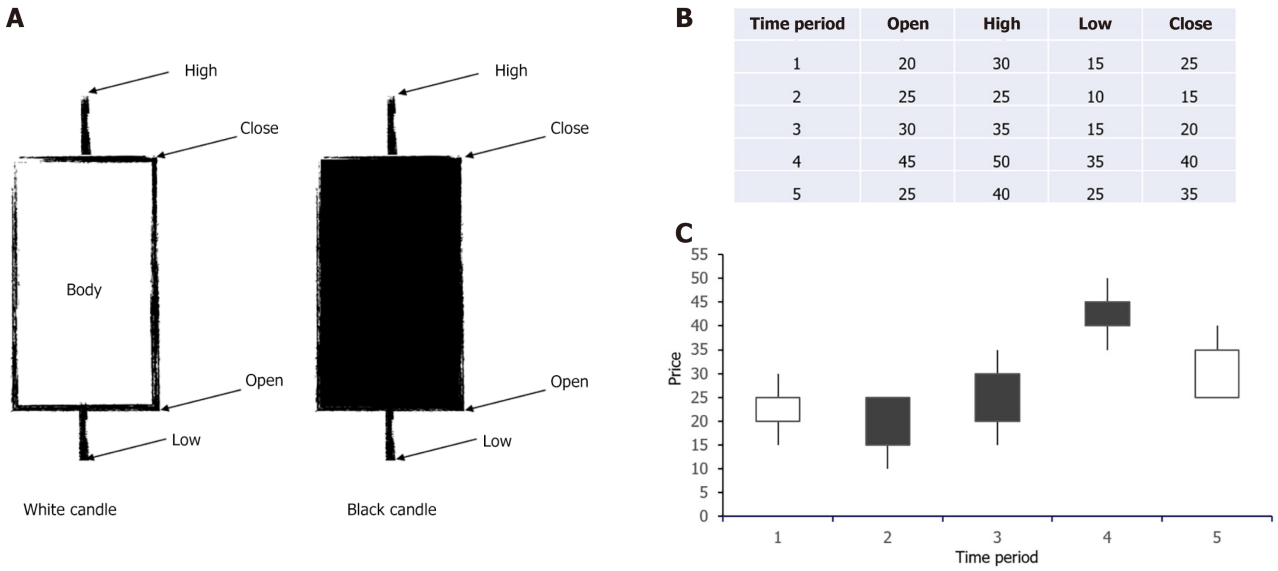
Information used to reflect the situation of a market through a candlestick chart is the opening, maximum, minimum, and closing price (Figure 1). Although other types of graphs called bar graphs use the same information, candlestick graphs are visually much more attractive, facilitating interpretation and analysis of data[14]. Similarly, to represent the daily glucose profile on a candlestick chart (daily summary candle), we would use fasting glucose, maximum glucose, minimum glucose, and bedtime glucose.

In a candlestick chart, the rectangle is called the body and represents the difference between the opening price, which would be equivalent to the glucose check before breakfast, and the closing price of the same day, which would be the last glucose check of the day before bed. The last glucose check is often informative to prevent the dreaded nocturnal hypoglycemia. Opening and closing prices are very important in Japanese candles and in the real life of diabetic patients.

The color of the Japanese candle indicates whether the market is bullish or bearish [*i.e.* if price (or glucose) has increased or decreased]. A white or green body means that the closing price was greater (higher) than the opening price; the patient goes to bed with a higher glucose level than when fasting. A black or red body means that the closing price was lower than the opening price; the patient goes to bed with a lower glucose level than when fasting.

Lines on the top and bottom of the body are called wicks, hairs, shadows, or highlights. They represent the maximum and minimum prices of the day or the highest and lowest glucose level that the patient has had. They allow detection of the maximum and/or minimum glycemic excursions very easily on a specific day.

After the close of the previous candle, a new one begins to form and the starting point is the closing level of the previous candle. That is the opening price of a Japanese candle. The closing price is the highest level of the body of the Japanese candlestick, if it is bullish. If it is bearish, it will be the lowest point of the body. From that level, the next candle



Time period	Open	High	Low	Close
1	20	30	15	25
2	25	25	10	15
3	30	35	15	20
4	45	50	35	40
5	25	40	25	35

Figure 1 Japanese candlesticks. A: White and black candles illustrated; B Time period, constructing the candlestick lines; C: Candle chart. Adapted from Nison [15].

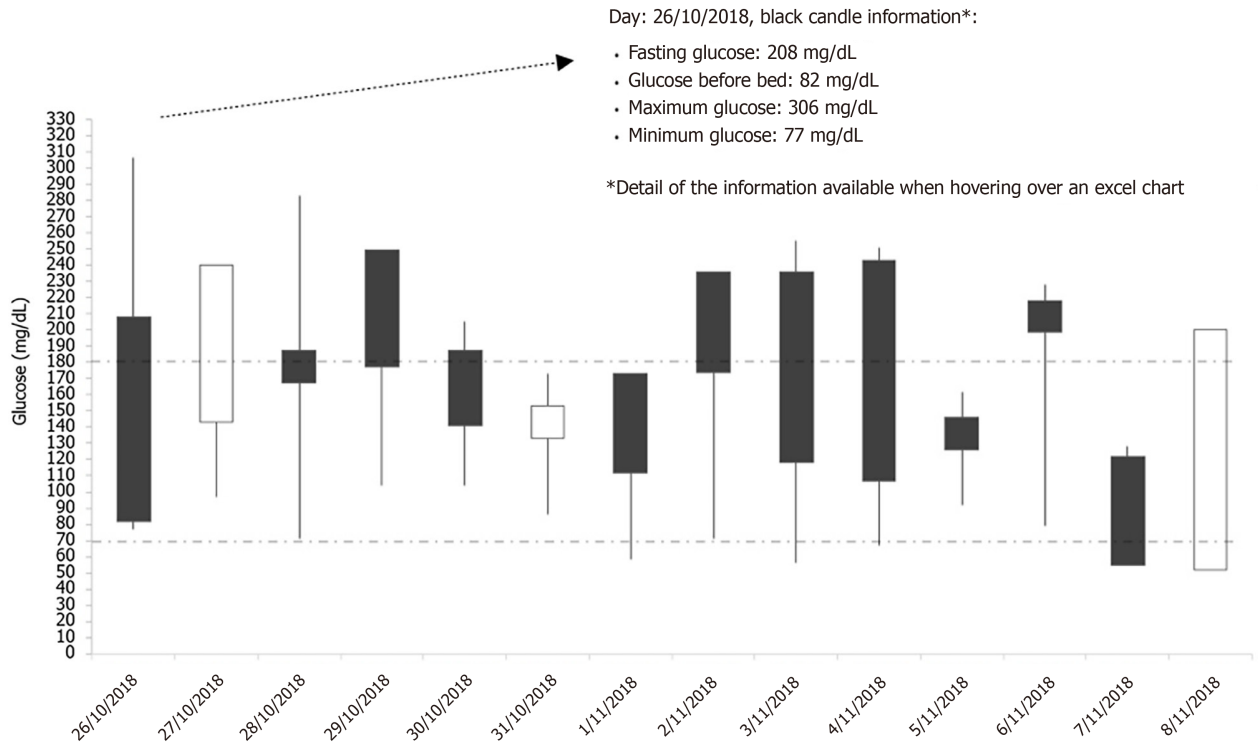


Figure 2 Candlestick chart for daily glucose profile.

begins. When the opening and closing prices are the same, they are called a doji candle.

As expert technical analysts evaluate the probability of a price reversal or a trend change, they prefer to rely more on patterns formed by two or more successive candles rather than by individual Japanese candles. In the same way, in diabetes we adjust insulin treatment after observing the evolution of glycemic control for 2-3 d, except in cases of risky glycemic excursions, in which we act immediately. To carry out a successful financial operation, waiting for confirmation from the pattern before carrying out any act of purchase or sale is required. This waiting rule applies even in cases where patterns predict market reversals with a high success rate. It is of the utmost importance to be patient until confirmation arrives. As we see, in the management of diabetes we proceed in a similar way.

Japanese candles can be used in any time frame (minute, hour, day, month). The longer the time frame, the less noise has been made. As we have seen, they allow the identification of trends through colors and maximum and minimum values.

Figure 2 represents a daily glucose profile represented by a candlestick chart for a time frame of 14 d. As can be seen, it is easier to quickly draw conclusions from the candlestick chart regarding glucose pattern and hypo/hyperglycemia (minimums and maximums) if there are any.

In the example, most of candles are black, which indicated that the patient went to bed with lower glucose levels than when she/he woke up. However, fasting figures are mostly above range. Most bodies exceed the recommended range. We also see that this patient suffered five episodes of hypoglycemia, with the lowest level of 52 mg/dL. Therefore, it can be concluded that glycemic control must be optimized and the tendency toward hypoglycemia prevented.

Candlestick charts could also be useful in the hospital where most patients with diabetes do not have CGM but are subject to intensive capillary glucose control. These charts quickly provide information on trends and daily lows and highs.

In conclusion, the use of the Japanese candlestick chart in the graphical representation of glycemic control, especially in the AGP report and in situations of intensive capillary glycemic control, should be considered. The Japanese candlestick chart can compare the current trend with previous periods and identify minimum and maximum glucose values at a glance as well as glucose patterns throughout the day. Collecting the "closing" glucose of the day should be useful to prevent nocturnal hypoglycemia.

FOOTNOTES

Author contributions: Boj-Carceller D conceptualized, designed, and wrote the report.

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Country/Territory of origin: Spain

ORCID number: Diana Boj-Carceller 0000-0002-6603-3193.

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LETTER TO THE EDITOR

Simulated patient methodology as a "gold standard" in community pharmacy practice: Response to criticism

Christian Kunow, Bernhard Langer

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Christian Kunow, Bernhard Langer, Department of Health, Nursing, Management, Neubrandenburg University of Applied Sciences, Neubrandenburg 17033, Germany

Corresponding author: Bernhard Langer, BSc, MSc, PhD, Professor, Department of Health, Nursing, Management, Neubrandenburg University of Applied Sciences, Brodaer Straße 2, Neubrandenburg 17033, Germany. langer@hs-nb.de

Abstract

The simulated patient methodology (SPM) is considered the "gold standard" as covert participatory observation. SPM is attracting increasing interest for the investigation of community pharmacy practice; however, there is criticism that SPM can only show a small picture of everyday pharmacy practice and therefore has limited external validity. On the one hand, a certain design and application of the SPM goes hand in hand with an increase in external validity. Even if, on the other hand, this occurs at the expense of internal validity due to the trade-off situation, the justified criticism of the SPM for investigating community pharmacy practice can be countered.

Key Words: Simulated patient methodology; Community pharmacy; Gold standard; Covert participatory observation; Internal validity; External validity

Core Tip: The simulated patient methodology (SPM) is considered the "gold standard" as covert participatory observation. SPM is attracting increasing interest for the investigation of community pharmacy practice. However, there is criticism that SPM can only show a small picture of everyday pharmacy practice. However, if the SPM is designed and applied in a certain way, this criticism can be countered.

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TO THE EDITOR

The simulated patient methodology (SPM) is a participatory observation in which simulated patients (SPs) covertly contact a healthcare facility and simulate participation in a seemingly real service process based on a predetermined scenario[1]. In both older and more recent international literature, the SPM is considered the "gold standard"[2,3], in part due to its high internal validity[3]. In addition to application settings such as health insurance companies, hospitals, and primary care, SPM also plays a major role in community pharmacies[4]. There has been an increasing number of SPM studies on pharmacy practice[4,5], such that not only worldwide reviews[4,5] but also reviews on selected regions[6] and even individual countries[7] have been published. In fact, other review articles - in addition to those already compiled on visits[4] as a "traditional"[8] form in the CP setting - are planned for other forms of SPM such as calls[9]. Finally, a checklist for reporting research using SPM (CRiSP) was recently published, based on a Delphi study focusing on pharmacy[10].

In addition to the increasing interest in SPM to investigate CP practice, there is also criticism that the SPM can only represent a small picture of everyday pharmacy practice[11] and therefore has limited external validity[3]. One of the reasons for this critique is that in a single contact with a CP, the service process of only one specific pharmacy employee can be observed and evaluated at a given time, whereby only one SP with certain characteristics can participate. Furthermore, the SP can either make a purchase for themselves (self-purchase scenario) or for a third party (purchase for a third-party scenario)[12]. Finally, the SP's demand may only include a specific product (*e.g.*, aspirin), condition (*e.g.*, migraine), or symptom (*e.g.*, headache) based on a corresponding simulated scenario[3]. Conversely, in everyday pharmacy practice, different pharmacy employees and customers as well as different scenario combinations can in principle be mapped at different times; however, this was not reproduced in the SPM studies examined in the reviews[4, 5], with a few exceptions[13].

To provide as comprehensive a picture as possible of everyday pharmacy practice, a certain design and application of the SPM are required. For example, a CP should be contacted several times and at different times to increase the probability of being able to observe and evaluate several pharmacy employees. SPs with different characteristics (*e.g.*, 20/40/60-year-old man/woman with/without migration background, *etc.*) should be used in coordination with the scenario (*e.g.*, an 80-year-old man's request for emergency contraception does not correspond to everyday pharmacy practice) to account for the diversity of customers. Finally, several scenario combinations should be used. Accordingly, an SPM study should include scenarios for self-purchase and purchase for a third party and (ideally) at least the conditions or symptoms most frequently responsible for self-medication, and in relation to these, the products most commonly requested or recommended in guidelines. On the one hand, such a design and application of the SPM goes hand in hand with an increase in external validity. Even if, on the other hand, this is at the expense of internal validity due to the trade-off situation and is also associated with considerable personnel and organizational effort, the justified criticism of the SPM for investigating CP practice can be countered.

FOOTNOTES

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Country of origin: Germany

ORCID number: Christian Kunow 0000-0002-2674-0344; Bernhard Langer 0000-0001-8410-5229.

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