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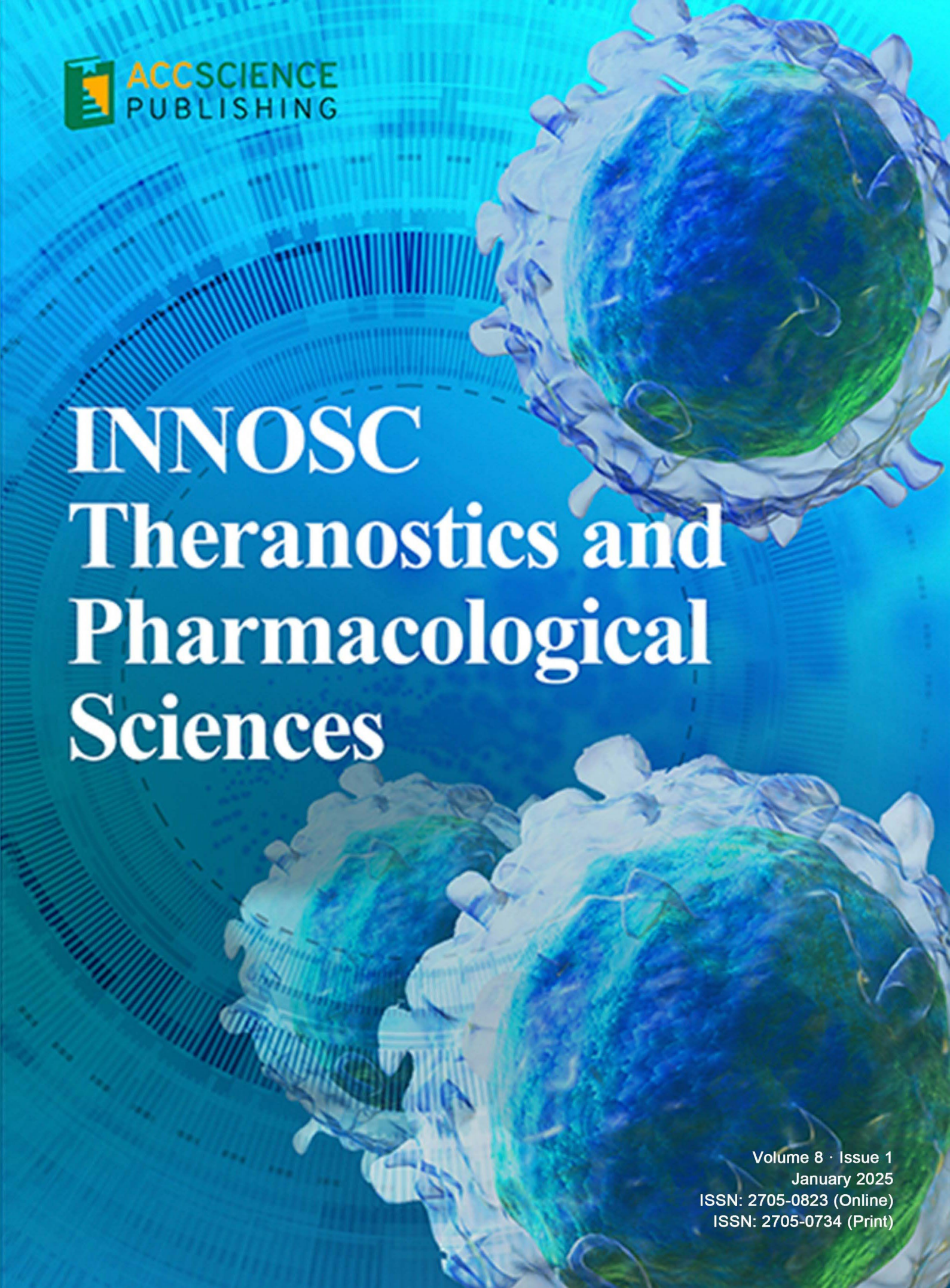

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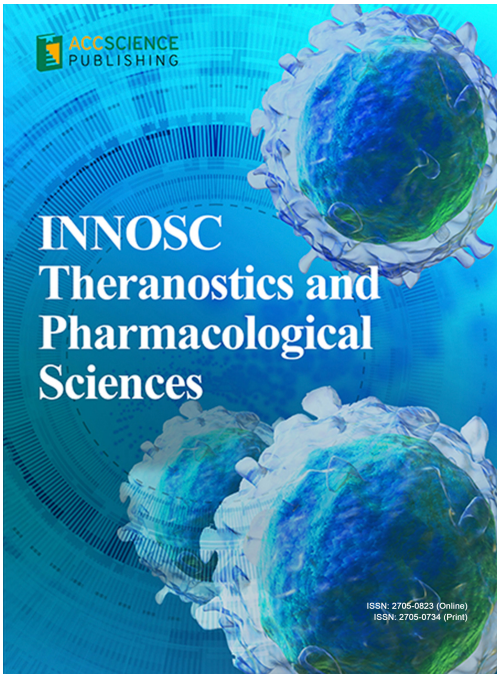

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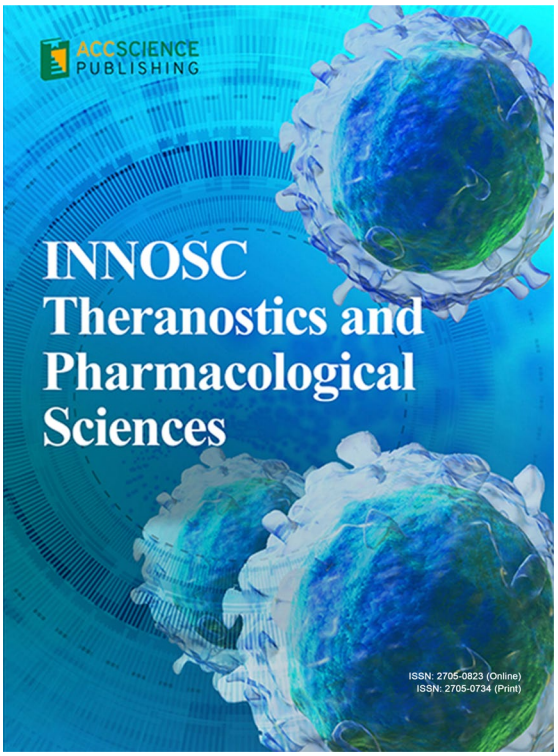

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INNOSC Theranostics and Pharmacological Sciences

REVIEW ARTICLE

Planning and performing image-assisted robotic interventions using personalized, minimally invasive, safe, and precise therapeutics

Adel Razek*

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Abstract

This review aims to analyze complex medical interventions planned and performed using image-guided robots. Such interventions, which may involve surgical or targeted drug delivery, are minimally invasive, precise, and safe therapies. The accuracy of robotic positioning is improved by reducing uncertainty and complexity, which can be achieved by matching real and virtual interventional procedures involving physical and virtual phantoms of the relevant part of the corresponding living tissues. Such tailored training includes personalized, patient, and interventional tool characteristics, and the results enable a real (with patient) intervention controlled by staff and a possible matched autonomous intervention under staff supervision. This paper discusses considerations for selecting appropriate scanners to control and monitor image-guided interventional procedures, planning personalized medical interventions using physical and virtual phantoms, involving staff in the loop, and employing augmented matched digital twins (DTs) for real interventions. Moreover, the paper positions the image-assisted robotic strategy in comparison to laparoscopic surgery. Each topic covered in this article, while self-contained, is supported by examples from the literature to facilitate a deeper understanding. The outcomes of this review highlight the importance of complex medical interventions involving image-assisted robotics or laparoscopic processes involving minimally invasive, nonionizing, and precise interventions. Furthermore, DTs already integrated into healthcare, combined with digital tools, could offer an effective solution for managing image-assisted robotics. This includes planning interventions with phantoms or patients and involving staff in the loop.

Keywords: Image-guided therapeutics; Living tissues; Surgery and drug delivery interventions; Complexity; Uncertainties; Monitoring paired real–virtual twins

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1. Introduction

Throughout history, the drive for progress has remained constant. Today, many routine processes and technologies contribute to modern well-being, with one of the most critical domains being health-related strategies that prioritize safety, comfort, and curative outcomes. Recent advances in medical sciences have uncovered the causes of numerous illnesses and developed strategies to address them, primarily through surgical and drug delivery interventions. The effectiveness of these interventions is directly linked to factors

such as safety, comfort, and curative outcome of the patient, which rely heavily on the tactile and visual skills of medical staff. These interventions aim to be minimally invasive and precisely targeted, thus protecting healthy tissues surrounding the affected areas. Furthermore, medical staff is expected to deliver personalized patient care, which intensifies the skills required for operational efforts. The concept of personalized medicine involves medical care tailor-made to the specific requirements of each patient, allowing for, in addition to the specific disease to be treated, factors such as hereditary characteristics, daily life, ecological environment, and reaction to treatments.

Based on the above-described information, the least invasive and positional precision tactile and visual skills could be advantageously replaced through dedicated image-guided robotics[1-4] or equivalent strategies such as laparoscopic surgery.[5] In fact, robotic surgery is the daughter of laparoscopic surgery with an evident improvement in technology and also in the fatigue of the surgeon who assumes a more comfortable position for the entire duration of the procedure. Such substitution is particularly further recommended for more complex interventions. Moreover, such interventional complexity that can be encountered during major surgical procedures[6-9] or limited drug delivery[10-12] obligates actions in a restricted area, as mentioned earlier, to protect healthy living tissues bordering the affected area. Hence, closed-loop robotic or laparoscopic processes would enable complex interventions reflecting positioning precision, minimally invasive, and restricted-area medical rulings by assistance using methods that are harmless to healthy living tissues.[4,5] In such closed-loop robotic control processes, different problems may be encountered, related to the management of complexity, uncertainty, and unanticipated dangerous incidents. Such problems can be solved using a control strategy based on matched physical–virtual pairs.[4,13] Therefore, the concept of personalized medicine as well as the last mentioned medical rulings could be planned (predetermined) by monitoring a matched physical–virtual pair (involving a physical phantom and its model) allowing the verification of the intervention result. This supervision can be aided by a human intercession. Furthermore, in expectancy, a monitoring of the real–virtual pair could be autonomous in the presence of the patient and with the medical staff in the loop.

The aim of this review is to analyze complex personalized medical interventions planned and performed using image-guided robotics, ensuring minimally invasive and safe conditions for precise surgical or drug administration procedures. The next sections in this paper are summarized as follows. Section two is dedicated to image-guided medical interventions involving features of adapted interventional scanners. Section three discusses the control and monitoring of image-guided intervention procedures comprising closed-loop control of image-assisted robotics and the reduction of perturbing factors in such control by monitoring real–virtual pairs. Section four discusses the planning of personalized and medical rulings, which incorporate both physical and virtual phantoms, along with integrating prospective individuals (staff) in the loop and using augmented digital twins (DTs) for real (with patient) interventions. A discussion on additional details of notions and concepts involved in the developed analyses is provided in section five. Finally, section six discusses conclusions and future suggestions.

2. Image-guided medical interventions

Traditional invasive interventions pose challenges in surgical procedures and restrict drug administration. At present, minimally invasive, image-assisted robotics enhance patient ease and safety as well as procedural accuracy and therapeutic efficacy. These methods can target almost any area in the body, and controlled release through implants prevents side effects.

2.1. Characteristics of interventional scanners

Most imaging scanners can be used in robotic assistance. However, each of the imaging methods is acceptable for an explicit condition.[14] Those engaging ionizing radiations, such as X-ray and positron emission, would not be appropriate for extensive-duration actions. Consequently, only the two nonionizing scanners, *namely*, magnetic resonance imaging (MRI) and ultrasound imaging (USI), would fit such prolonged actions.[15-18]

An imaging scanner is expected to provide high-resolution, 3D visualization the tissue structure and close-action instrumentation. Robotic assistance works within the imager scaffold alongside the object, enabling closed-loop management of tasks, such as tracking object motion and distortion, tracing robotic tools, and controlling drug release. Due to the increasing use of interdependent scanner–robot actions, a new approach has emerged that allows medical staff to treat patients more efficiently. Placing robotics within the imaging scaffold merges imaging capability with robotic competence, thus developing closed-loop management.

2.2. Features of MRI and USI

Both MRI and USI exhibit the abovementioned features relative to imaging and interventional tasks. However, USI can function only in boneless and airless frames.[3,4] The second distinction is the MRI's requirement for a scaffolding environment devoid of electromagnetic (EM) noise.[19-25] However, MRI appears to be a comprehensive scanner conditional to circumventing EM noise.

In addition to these distinctions, other applications can be identified. MRI is suitable for imaging entire body parts without constraints. Although MRI is universally applicable, USI can serve as a replacement when appropriate for the object being examined. Moreover, USI offers good maneuverability and cost-effectiveness, whereas MRI, although capable of producing high-quality images of soft tissues, is more complex and expensive. Therefore, from the viewpoint of practical use, the choice between the two scanners depends on the situation. USI should be used whenever possible, whereas MRI should be reserved for imaging structures containing bone and/or air, such as the brain and certain other body parts. Typically, clinical centers that perform MRI-guided procedures likely possess surgical imaging facilities, eliminating the need for scanner transport. In terms of cost, when patient well-being is a consideration, MRI-guided interventions, such as brain surgery, are the only nonionizing, minimally invasive treatment options.

In fact, MRI can provide high-level 3D images of the object configuration, neighboring healthy tissues, and involved instruments; however, there are significant challenges in its implementation to successfully guide the intervention. These challenges include the use of three magnetic fields of different characters (magnitude and frequency), presenting allergic responses to EM noise, and imposing a restricted occupation zone inside the imaging structure. The latter problem can be overcome using an open scanner, although it involves tolerating the disadvantage of lower field intensity and therefore a slower process. Nonetheless, MRI may be better than other scanners for several reasons. It has an incomparable contrast allowing the visualization of tumors as well as other abnormalities undetectable by other scanners. It has a true 3D imaging capability, including multimodal detection, *for example*, blood flow, temperature, and biomarker tracking. In these circumstances, the training of robotic assistance by an MRI can allow an excellent intervention.

3. Control and monitoring of image-guided interventions

Intraoperative imaging strategies have created a need for medical tools that address the requirements of different imaging techniques. Developments in image resolution and disconnection capabilities have enabled new interventions. 3D imaging techniques provide realistic and detailed views of living tissues; however, the tool must be activated within a specific area by locating the image coordinates. Moreover, operative drug delivery procedures can deliver drugs to the target site, maintaining therapeutic drug levels with minimal impact on surrounding healthy tissues. This can be facilitated by drug-delivery implants.

3.1. Closed-loop control of image-assisted robotics

As mentioned earlier, for patient comfort and safety, therapy must be limited to the affected area during surgery or drug delivery. Such precision depends on the accuracy of tool actuation and space localization. Accordingly, the prerequisite for such high-performance topological tracking is image-supervised location identification. Such conditions require a collaborative organization working autonomously, as demonstrated in a controlled drug release setting (Figure 1). This system includes the scanner, tissue-affected area, drug delivery implant, control system, and drug supply.[3,4]

The accuracy involved in such a control process related to actuation and spatial localization would be influenced by different disturbing factors, including the degree of complexity of the components of the combined procedure, the associated detection uncertainties, and different unanticipated external hazard incidents. Reliable control could be certified only after reducing these disturbing factors.

3.1.1. Surgical interventions

Image-assisted or laparoscopic intraoperative interventions that meet safety requirements for imaging and interventional actions generally use MRI and USI scanners[3,4,6] or laparoscopic strategies.[5] However, in some cases, MRI scanners are increasingly used in surgical interventions increasingly, particularly for their superior ability to differentiate tumors from normal tissues during surgeries and tumor extractions.[26-29] As mentioned earlier, MRI is sensitive to EM noise; thus, all robotic materials inserted into the scaffold near body tissues must be MRI-compatible (free of magnetic and conductive materials). In such settings, an actuation action is necessary. Few high-performance actuators are MRI-compatible. One possible class of actuation devices is piezoelectric actuators, which come in different categories. More information on their structures, materials, fabrication, testing, and applications is described elsewhere.[30-39] These devices must be tested to confirm their compliance, *that is*, to ensure that they do not disturb the field distribution produced by the radiofrequency (RF) coil used for imaging. Such devices are composed of piezoelectric materials, which are dielectrics and thin electrodes. Although dielectrics are not expected to disturb the field, conductive electrodes, even those of minimal size, must be carefully tested and regulated.

3.1.2. Drug delivery interventions

Conventional drug administration by intermittent oral or intravenous release can cause extraordinary blood drug concentrations immediately after delivery, probably

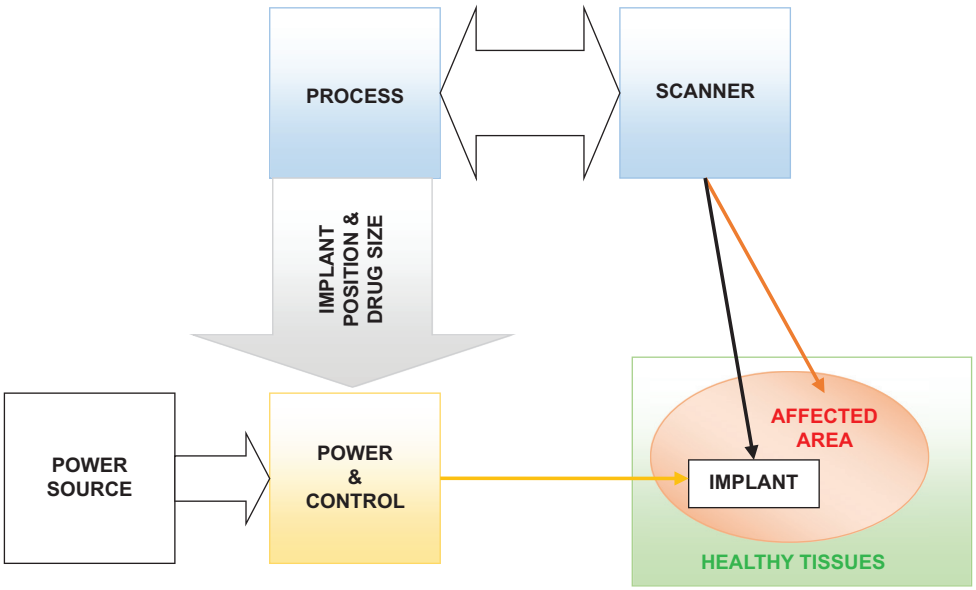

Figure 1. Schematic of an autonomous restricted drug release setting comprising the scanner, tissue affected area, drug delivery implant, control system, and supply

resulting in a dangerous effect in patients. Moreover, these methods undergo first-pass metabolism, causing a substantial concentration decrease in the liver before attaining regular flow, and consequently several doses are often required. Local, sustained, and controlled delivery allowing less hostile effects is possible through implant technology. Two situations regarding the spatial release are concerned. The first is spatially regular with constant drug release and biodegradable static configurations that enable their disposal; more information concerning the structures, materials, composition, and characterization is described elsewhere.[40-50] The second is spatially non-uniform centering only on sick zones and circumventing healthy zones; hence, mobile implants are necessary. The strategy used in the second case is similar to the case of image-assisted surgical interventions discussed in the previous section and satisfies safety features regarding imaging and interventional actions; these implants can involve different cases depending on the required precision, the treatment type, and the concerned placement.[12,51-58]

3.2. Reduction of control-perturbing factors

As mentioned earlier, the accuracy involved in the control process related to actuation and spatial localization is subject to disturbing factors, which include the degree of complexity of the components of the combined procedure, the associated detection uncertainties, and different unforeseen external hazard incidents. It is essential to reduce these possible perturbations and consider personalized patient data, which could be achieved by monitoring such factors and data through matching in physical–virtual pairs as DT tools.[59] DTs could be termed as a simple incorporation of data between a real phenomenon and its digital copy pair, in both ways. DTs are generally used for monitoring procedure conditions, diagnostics, prediction, optimization, scenarios, and risk evaluation.[60] Such tools can be designed at the levels of system, subsystem, and specific components. The concept of DTs is used for the administration of complex processes such as image-assisted robotized interventions; we describe here its central features.[61] A DT is organized as a real–virtual pair that permits self-adapting conduct. The real part provides the processed detected data to the virtual part, and the latter conveys the control instructions to the real part. Such a matching also allows reducing uncertainties and decreasing undesirable and menacing operating singularities.

3.3. DT management

Following the description of the primary features of a DT, this section describes in detail the management of its monitoring. The processed information of the real side provides sensor detections paralleled and amended by outward data (IoT) as well as by acquired history. The resulting product is communicated after training in a data analysis format. These submissions, with a suitable reduced model, are transferred to the virtual side. Rapid matching requires a reliable virtual replica with short computation times, which can be achieved by reducing the comprehensive model – *that is*, a complete coupled model closely resembling the real system – while maintaining the real physical picture. Administration using such a

pair consents an adaptive control for a procedure when functioning.[62] Figure 2 summarizes the topographies of a DT for monitoring an image-guided intervention procedure.

Note that the DT concept is progressively incorporating the healthcare sphere exploiting virtual replicas of bodily individuals that go further than a static situation incorporating the dynamic conduct of a real living tissues entity, many examples have been published in this intention, see for instance.[63-73] As the healthcare sector is gradually exploring the use of DTs, successful proofs of concept in various sectors suggest that, if fully realized, DTs could transform connected care, lifestyle, health, and chronic disease management in the future. However, recognition of the vast capacity of DTs in health care may likely be slowed by hesitation challenges.

4. Planning of personalized and medical rulings

The concept of DT discussed in the previous section could be used in planning a fictive intervention without a patient. In this case, we monitor the fully imaging-assisted robotic intervention procedure, including the scanner, the robot, and the tissue (phantom). This physical phantom of the tissue part of interest will be incorporated into the real physical side of the DT. Similarly, the virtual model side of the DT involving the intervention procedure model will include a digital phantom replica of the physical one. This corresponds to Figure 2, with physical and digital phantoms representing the body part of interest in the living tissue. This planning would include personalized characteristics of the patient and the interventional tool as well as the recommended medical prescriptions to meet the safety features related to imaging and interventional actions for the intervention concerned. Such planning allows confirming the smooth running of the intervention and the possible adaptations required for the medical protocol practiced. Therefore, the real (with patient) image-guided intervention could be performed under good conditions, which could be achieved through an image-assisted collaborative environment, as illustrated in Figure 1, under the supervision of medical staff, or prospectively through an autonomous DT monitoring procedure with involved staff (in the loop).

4.1. Physical and virtual phantoms

Physical and digital phantoms, discussed in the previous section, represent living tissues on both sides of the DT. A major problem with both choices lies in the way of tissue representation. Considering that biological properties allow the erection of these phantoms with static behavior, such a static representation could be suitable for specific parts of body tissues and for particular demands. Nevertheless, as mentioned earlier, these phantoms should represent real body tissues with reasonable biological properties. In general, the mechanical behavior related to the deformation and displacement of living soft tissues, irrigated by fluids allowing their functioning, corresponds to an exhibition of a complex dynamic behaviour.[74-78] Such a complex tissue behavior requires actually evaluated constitutive rules, adapted computational approaches, or both to address this problem. We can only approach this category of representation in an approximate manner. Different tissue models can be found in the literature.[79-82]

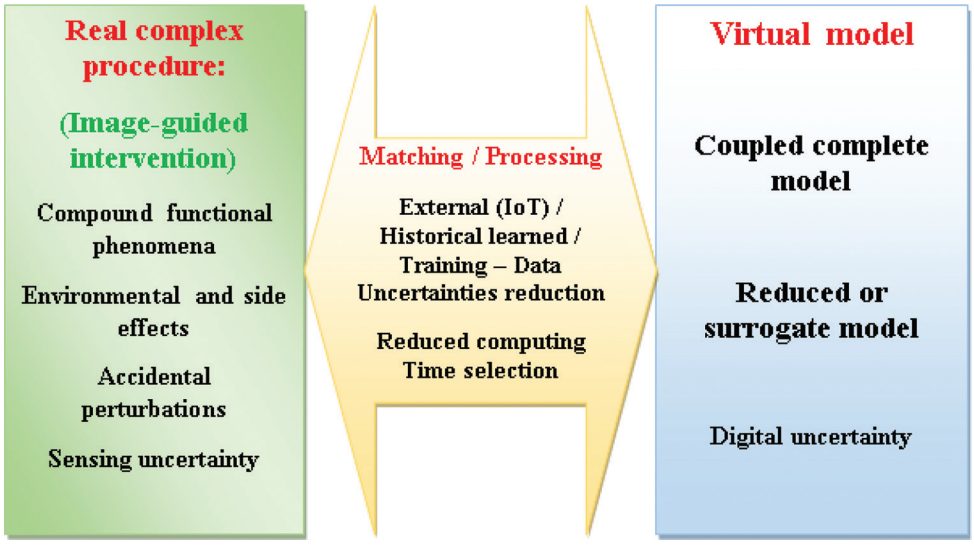

Figure 2. Summarized illustration of a matched monitoring of a complex procedure (image-guided intervention) with its virtual model

4.2. Prospective human in the loop and augmented DTs

The abovementioned human-robot alliance (staff in the loop) related to DT authorizes higher supervision of image-guided interventions, thus reducing the threat for the patient and ensuring a guaranteed outcome for medical staff.[83-85]

Furthermore, the commitment of the practices of artificial intelligence (AI) in such medical therapeutics helps in reducing the complexity of data acquirement and post-processing in MRI through strategy acceleration and providing quicker analysis periods with relaxed image processing.[86,87] AI can also be exploited to perform planned repeated training duties in image-assisted robotics related to the objective of the present paper.

The procedure can be significantly enhanced through augmented human-robot interactions, advancing the overall system with augmented reality (AR)-aided robotic actions. When combined with MRI, AR can reduce risks in complex procedures, such as tissue damage, bleeding, and post-operative trauma. Moreover, DTs can play a significant role in AR-supported robotic procedures. This allows for precise identification of the source of the disorder and the necessary action, using individual patient modeling from deep learning databases. Furthermore, the AR–DT combination enhances accuracy in suturing, knotting, and placement compared to manual operations.[88-92]

5. Discussion

In previous analyses, image-guided interventions have been comprehensively discussed and illustrated. Different necessary concepts or notions have been mentioned and used in these developments, which include complete coupled and reduced models, complexity, and matched real–virtual pairs. These aspects are further developed (extensions, reflections, or connected matters) and discussed in the following points:

- Historically, the concerns related to the minimally invasive surgical approach can be summarized as follows. Since the beginning of the 19th century, open surgery through laparotomy was practiced until the middle of the 20th century based on the argument that "you could see better with open surgery." Then, laparoscopic minimally invasive surgery revolutionized the approach to specific areas of the human body by providing an enlarged view through a tiny video camera and light at the end of a thin tool. Robotic image-assisted surgery is a successor to laparoscopic surgery, offering significant technological advancements and reducing staff fatigue by allowing them to maintain a more comfortable position during the procedure. An intervention, such as partial gastrectomy, whether performed using open, laparoscopic, or robotic image-assisted techniques, involves the same surgical duration, with only the access being different.
- A complex procedure involves several compound-interacting components characterized by different phenomena interacting in an interdependent manner, which is linked to the temporal and spatial behaviors of the involved phenomena. The closer the time constants and the higher the matter local nonlinearities in phenomena, the stronger their interdependence and, consequently, the greater the complexity. For distant time constants and linear matter behaviors, this interdependence and related complexity are greatly reduced. The concept of complexity is present in various natural and artificial occurrences[93] as well as societal issues.
- A complex procedure can be modeled mathematically considering its compound-interacting components through the coupling of the equations governing the different phenomena interacting in an interdependent manner. The more complex the procedure and its related phenomena interdependence, the more will be the complexity of the coupled model. For stronger phenomena interdependence (near time constants and nonlinear matter behavior), the equations would be strongly coupled (solved simultaneously). In the case of low interdependence (distant time constants and linear behavior), the equation coupling would be weak (iterative solution). Furthermore, the solution of the concerned equations should account for diverse topographies of modeled assemblies, which reflect geometrical complexity, variables nonlinear behaviors, and interdependence of phenomena. Satisfying such characteristics inflicts a local solution in the matter suggesting the practice of discretized 3D methods as finite elements (FEM) or comparable methods. More details regarding the types of discretized elements, domain meshing, methods mixing, and numerical methodology are described elsewhere,[94-103] accompanying appropriate strategies for equations coupling, with more details described elsewhere.[104] These coupled solutions would faithfully represent the behavior of the complex procedure. However, they reflect an important computation time and could not be suitable for real-time executions.
- In the case of repetitive or online real-time computations, complete coupled models would be penalizing, and hence reduced models should be used. Reducing a model involves hastening its solution while debasing its accuracy as slight as possible; more details are described elsewhere.[105] The trouble

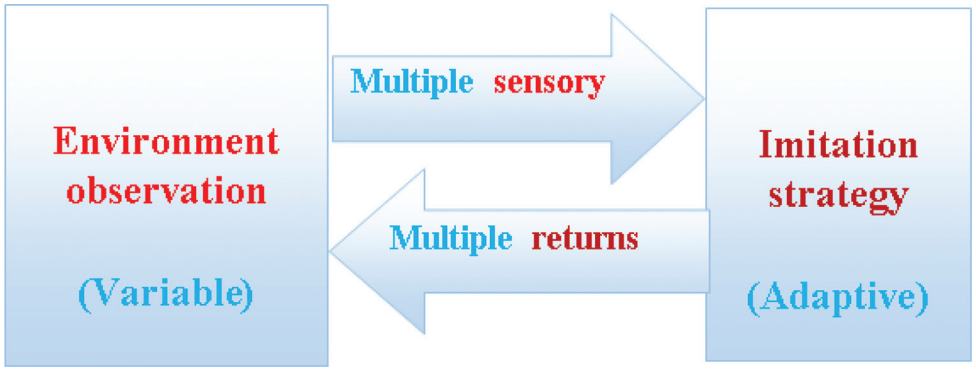

Figure 3. Schematic of camouflage involving the environment observation-imitation strategy pair with its two-way link (sensory–return) for variable environment real-time adaptive matching

is then frequently to obtain the border between sparing time and weakening exactness. The key idea originates from the observation that a superior model is much time-consuming to execute in real time" or consider revising suitably. A model reduction (surrogate model) is therefore substituted[106,107] for the superior model to perform a pre-sizing. Moreover, one can practice stochastic non-intrusive methods (*e.g.*, kriging and polynomial chaos)[108,109] that expend 3D FEM calculations with a restrained group of attainments (learning samples), thus providing efficient metamodels.

- Real-virtual matched pairs are materialized in this review article by the DT concept. This concept was first introduced by Michael Grieves in 2002 [62], although its principle existed earlier. For example, its use by NASA for complex simulations to control the safety of spacecraft following a worrying explosion in the oxygen tank of the Apollo 13 mission in 1970. After this accident, the mission adapted simulators for the real spacecraft conditions; this was possibly one of the first practical applications of a DT, although it was not a common concept in 1970. Moreover, the practice of real–virtual matching is related to virtual modeling or reasoned deduction accounting for the observation of an object or phenomenon. Hence, the matching (or mirroring) of an observable with its virtual image has been and still practiced in numerous natural and artificial circumstances. Humans and other living beings often rely on observation and sensory maneuvers, using deduction and mimicry to ensure self-protection and survival. Deductive reasoning, coupled with observation, is one of the earliest learned natural abilities. For example in wildlife, life security is based on observation, and occurrences of imitation strategy are recurrent, this is materialized by camouflage.[110] This facilitates living beings to blend into their environments through adaptive matching.

Figure 3 illustrates a representation of camouflage, highlighting the link between environmental observation, mimicry strategy, and its bidirectional (sensory–feedback) link for a variable environment using real-time correspondence.

6. Conclusion

This review paper contributed to the analyses of complex medical interventions involving image-assisted robotics (or laparoscopic) processes under conditions of minimally invasive, nonionizing, and precise surgical or drug delivery interventions. The adaptation of scanners for robotic interventions, their control and monitoring, the mitigation of perturbing factors, and the planning of personalized medical decisions with prospective augmented routines have been analyzed and discussed. DTs, which can be readily integrated into healthcare, may offer an efficient solution for managing image-assisted robotics when combined with digital tools. This can be implemented in various forms, including planning and interventions using phantoms and patient involvement, with staff in the loop. A potential future research direction could focus on further exploring the mechanical behaviors of living tissues in real time.

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Author contributions

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Availability of data

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ACCSCIENCE PUBLISHING

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REVIEW ARTICLE

Exploring the transcriptomic landscape of rheumatoid arthritis using next-generation RNA sequencing

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Abstract

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by inflammation and joint damage. Transcriptomics has been utilized in RA profiling to identify locations that were previously unclear. A robust technique for examining the intricate transcriptomic landscape of RA is next-generation RNA sequencing (RNA-seq). This review has explored the use of RNA-seq to understand the pathophysiology of RA. Detailed discussions are presented on the fundamentals of RNA-seq technology, how RNA extracted from the synovium is incorporated into gene expression patterns analysis, and how RNA-seq is superior to other methods. We have explored several applications of RNA-seq in RA research, emphasizing its potential for identifying dysregulated pathways, detecting novel biomarkers, and characterizing gene expression levels. Furthermore, this review has clarified the regulatory networks and signaling pathways identified in RNA-seq research and investigated the possibility of using transcriptomic RNA-seq as a diagnostic tool for RA. In conclusion, we have highlighted the significance of using transcriptome data to achieve a more comprehensive understanding of the molecular processes underlying RA. Hence, this review underscores the revolutionary influence of RNA-seq on RA research, paving the way for improved diagnostics, tailored therapy, and the discovery of novel treatment targets for individuals with RA.

Keywords: Rheumatoid arthritis; Next-generation RNA sequencing; Long non-coding RNA; Circular RNA; Transcriptomic study

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1. Introduction

Approximately 0.5 – 1.0% of people worldwide suffer from rheumatoid arthritis (RA), a prototypical chronic inflammatory rheumatic disease that is affected by various hereditary, epigenetic, and environmental factors. RA is a prevalent autoimmune disease that affects the entire body and is characterized by significant joint dysfunction and persistent inflammation. The source of the original term "rheumatoid arthritis" indicates inflamed and fluid joints. Although RA primarily affects the joints, it may impact other organ systems such as the eyes, skin, lungs, liver, heart, and bones. Symptoms of RA often include pain, stiffness, fever, edema, and inflammation in the afflicted joints. These symptoms generally affect the small joints in the hands and feet; however, they can also

affect larger joints such as the knees and shoulders. Increased stiffness in the morning is a prominent symptom of this condition, and these symptoms become more noticeable after extended periods of inactivity.[1-3] RA-related pain is categorized as nociceptive rather than neuropathic because it originates at the site of inflammation. As the pathological state worsens, continued inflammation causes tendon binding, erosion, and degradation of the articular surface; this can limit the range of motion and cause deformity and local osteoporosis, which frequently develops around the inflamed joints of patients with RA.[4] Rheumatoid and musculoskeletal disorders represent a significant global economic burden, and their prevalence is increasing because of changes in behavior and population; among them, RA is the most prevalent inflammatory arthritis. As these disorders cause more functional impairments in adults than any other ailments, they are considered the primary cause of disability and early retirement among workers. Therefore, early identification and individualized, cell-specific therapies are essential for the management of RA.[5]

1.1. Pathophysiology of RA

In RA, the synovium is essentially inflamed, which causes erosion of the bone and cartilage, as depicted in Figure 1. Although the precise cause of the inflammation remains unknown, macrophages, which are often found in the synovium, are known to secrete cytokines such as tumor necrosis factor-α, interleukin-1, and interleukin-6, which cause inflammation. These cytokines stimulate fibroblast-like synoviocytes (synovial intimal cells are believed to be in charge of producing synovial fluid components), which start multiplying upon activation.[6] Simultaneously, synoviocytes support the expression of RANKL (osteoclast differentiation factor), which, in conjunction with cytokines, increases osteoclast activity (bone degradation), ultimately resulting in bone erosion.[7] Proteases are secreted by activated, proliferating synovial cells and tissues similar to fibroblasts. These proteases lead to cartilage deterioration or breakdown. Activation of fibroblast-like synoviocytes results in symmetrical arthritis. T cells can release interleukin-17, which activates fibroblast-like synoviocytes and increases macrophage activity; moreover, these cells induce inflammation.[8] Furthermore, T cells aid in the expression of RANKL, which activates osteoclasts to cause bone degradation. Moreover, plasma cells are present in this region; although plasma cells constitute only 5% of the overall immune cells, they support inflammation by producing cytokines and antibodies. Neutrophils are also present in the synovial fluid and secrete proteases and reactive oxygen species, which degrade cartilage and bone.[5,8]

An attribute of RA is the presence of immune complexes in the synovial fluid, which are composed of bonded antibodies that increase inflammation. All these cells release cytokines, which aid in increasing capillary permeability and adhesion molecule expression on the vascular vasculature, enabling immune cells to travel into the joint and induce RA, as shown in Figure 2.[9]

RA can be caused by factors such as smoking, heredity, and the bacterium *Porphyromonas gingival* that causes gingivitis. These factors can alter autoantigens, resulting in joint inflammation and cytokines. Antigen-presenting cells recognize this change and initiate an immune response. CD4 T-cell activation triggers B-cell activation in the germinal center, leading to the formation of plasma cells that generate autoantibodies against the body's own antigen.

An important component of RA is antibodies. Rheumatoid factor IgM antibody targets the Fc region of IgG and is present in 75% of patients with RA, as well as anticitrullinated protein antibodies, which target citrullinated proteins such as fibrin and filaggrin. These antibodies also cause inflammation and synovial fluid deposition. Proteins in which their arginine residues are citrullinated are known as citrullinated proteins. After the attack, the body targets this protein, recognizes it as a foreign particle, and begins to attack, causing swelling and inflammation.[5]

1.2. Clinical manifestation

RA is a symmetrical arthritic joint with pain, swelling, and nodules. Early symptoms affect the hand, particularly the proximal and metacarpal phalangeal joints. As the disease worsens, additional hand characteristics such as swan neck, Boutonniere, and thumb Z deformity appear. Angiogenesis, the expansion of blood vessels from pre-existing vasculature, is also observed in patients with RA.

2. Overview of transcriptomics: Understanding RNA sequencing (RNA-seq) technologies

Genomes in organisms are composed of DNA and genes, which remain almost identical in all cells. Nevertheless, when an organism experiences specific environmental changes, its cells must decode this genomic information differentially. This information is translated into RNA, which is used to produce proteins. The transcriptome is a complete set of messenger RNA (mRNA) transcribed in a cell at a specific time, expressing a part of the functional genome. Transcriptomic studies examine the transcriptome using various techniques.[10]

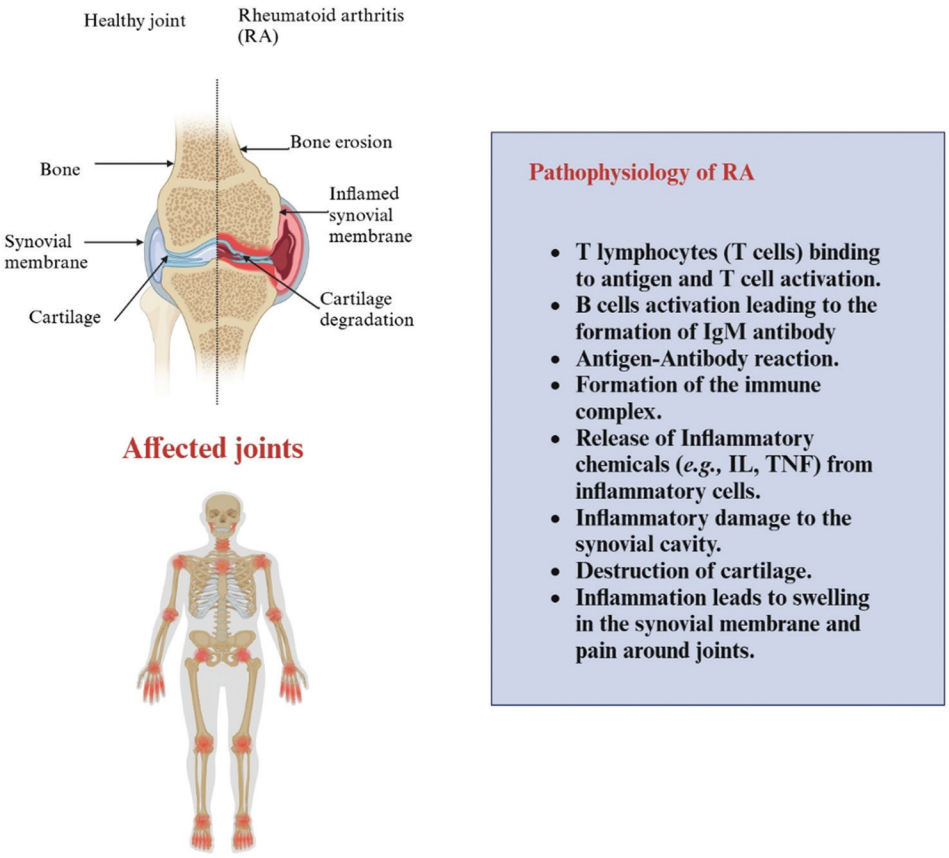

Figure 1. Pathophysiology of rheumatoid arthritis. Figure created by the author.
Abbreviations: RA: Rheumatoid arthritis; IL: Interleukin; TNF-α: Tumor necrosis factor-α.

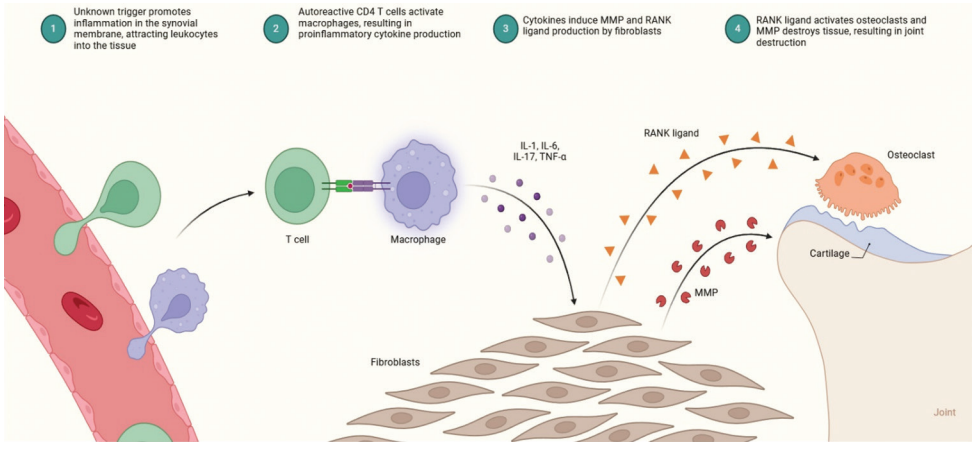

Figure 2. Pathogenesis of rheumatoid arthritis. Figure created by the author.
Abbreviations: IL: Interleukin; TNF-α: Tumor necrosis factor-α; MMP: Matrix metalloproteinase; RANK: Osteoclast differentiation factor.

The DNA microarray or chip method is based on nucleic acid hybridization. In microarrays, which are also known as cDNA arrays, a pre-determined set of probes representing the sequence of the gene fragments are fixed on a solid chip. A major limitation of this technique is that we must be aware of the gene sequence for designing the chip. Hence, for those species whose genomic sequence information is unknown, microarray is not a good option because chips are not readily available and the manufacturing of new chips is expensive. Another issue

with this array is cross-hybridization. Using full-length cDNAs, the mRNAs of paralogs can cross-hybridize to the wrong spots. A major challenge in analyzing microarray data is that it can be difficult to compare transcripts.[11]

Another method is a sequencing-based technique or RNA-seq. This technique largely replaces the microarrays, becoming popular for performing transcriptomics. The development of nanotechnology enables the sequencing of hundreds of thousands of DNA molecules simultaneously. RNA sequencing is a process for determining the nucleotide sequence of an RNA molecule in a specific sample. The primary principle is based on a high-output sequencing technique with a computational technique to capture and quantify transcripts present in an RNA extract. The nucleotide sequences generated are typically approximately 100 bp in length, but they can range from 30 bp to more than 10,000 bp, depending on the sequencing method used. In this method, RNA can first be isolated from biological samples and converted into double-stranded DNA molecules that serve as a template for DNA polymerases during next-generation sequencing (NGS) library preparation. These DNA copies are known as complementary DNA (cDNA), and the generation of cDNA is initiated using the reverse transcriptase enzyme and priming to form the first strand of cDNA.

The second strand is synthesized using DNA polymerase, leading to the formation of double-stranded cDNA. In the next step known as adapter ligation, the adapters ligated to the cDNA fragments and the resulting library molecules are amplified and sequenced.[12] In this method, short-read sequencing and map construction of the reads using a reference genome are performed using tools such as HISAT2 or STAR, and novel transcripts are assembled using tools such as StringTie. Other tools such as HTseq help in estimating the expression levels of the transcripts. Three factors, namely transcript level, transcript length, and sequencing depth, affect the number of read transcripts that can be detected in a sample. RNA-seq does not require prior genomic information, which makes it suitable for analyzing transcriptomes in non-model species.[13,14] Transcriptome analysis is generally performed for two major purposes; one is qualitative estimation, that is, identification of genes and their isoforms, and the other is quantitative analysis, that is, estimating the gene expression or relative expression in different biological samples.[15] RNA is less stable than DNA; however, the transcriptome is considerably more cell- and tissue-specific than the genome and is crucial for the generation of high-quality data.[16] Table 1 shows the transcriptomes used for RNA-seq to demonstrate the relevance of the approach across several conditions beyond RA.

Human leukocyte antigen (HLA) genes and non-HLA genes are the genes and genetic loci that have been linked to the risk of RA. RNA-seq sheds insights into the causes of RA and its therapy, making it easier to distinguish healthy individuals from those with RA. Studies[27,28] comparing population transcriptomes by analyzing the synovium, synovial cells, white blood cells, and peripheral blood mononuclear cells have been conducted. Studies have also revealed that patients with RA have dysregulated levels of secretory phosphoprotein 1 (SPP1). Inflammation, T-cell activation, and apoptosis are caused by SPP1. Proteoglycan 4 is detected in the severe stage of RA. According to some studies, a correlation exists between the expression levels of transcripts linked to hematopoiesis, interferon-mediated immunity, and cell trafficking and the advancement of arthritis.[27,28]

3. Application of next-generation RNA sequencing (RNA-seq) in RA research

The cutting-edge method known as "next-generation RNA sequencing" is used to analyze transcriptomes and provides extremely high yields by determining the nucleotide order in certain genomes or targeted DNA or RNA sections. The discipline of RNA sequencing is revolutionized by NGS. The three primary benefits of NGS are its cost-effectiveness, high throughput, and precise RNA characterization. The current research using RNA-seq for RA theranostics is presented in Table 2.

3.1. Novel biomarker discovery

Biomarkers are biological substances found in bodily fluids such as blood and tissues that indicate the presence of a disease. Some biomarkers for RA include rheumatoid factor, anticyclic citrullinated peptide, erythrocyte sedimentation rate, and C-reactive protein. Imaging biomarkers such as X-rays and MRIs can detect joint erosion and synovitis. Genes such as SNPs and HLA-DRB1 can also function as biomarkers, helping to determine the severity and course of a disease. Elevated levels of chemokines and cytokines during the proinflammatory phase can also be detected in certain patients with RA.[29-32]

3.2. Identification of dysregulated pathways

RNA sequencing helps in determining the RA pathways. RNA-seq facilitates the determination of transcriptome abundance levels and allows for the comparison of gene expression patterns with those of a healthy individual. The genes that are dysregulated help in indicating the molecular pathways.[23]

3.3. Gene ontology

Gene ontology helps in the processing of RNA-seq raw data. It involves quality control to remove any reading of

Table 1. Transcriptomic RNA sequencing across several conditions

S. No.	Condition/ disease	Study pattern	Methodology adopted	Transcriptome profile	RNA sequence analysis and database	Findings	Year of study	References
1	Alzheimer's disease (AD)	This study successfully produced transgenic mice expressing IE2 in the hippocampal region, revealing that IE2 induces the expression of a gene related to disease-associated microglia.	PBS was administered to the mice before hippocampal tissue removal, and instructions were provided for constructing a 3′-library. Barcoded data were demultiplexed and mapped to the mouse genome.	Differentially expressed genes (DEGs) affecting the transcriptome profile were discovered in the microglia of IE2 transgenic mice, including downregulated and upregulated transcripts such as Tmem119, P2ry12, ApoE, CD83, and ISG15.	The sequencing data were analyzed using Cell Ranger or 10× Genomics Cloud Analysis through the Loupe Browser.	The study's successful development of IE2 transgenic mice presents a novel approach for investigating HCMV infection *in vitro*.	2024	17
2	Autoimmune hepatitis (AIH)	This study aimed to investigate the potential molecular pathways related to AIH and describe the characteristics of peripheral blood mononuclear cells (PBMCs) in AIH using single-cell RNA sequencing (scRNA-seq).	The study used scRNA-seq to produce single-cell transcriptomes from healthy controls and patients with AIH, revealing differential expression profiles and cell clusters to understand the biological roles of AIH immune cells.	In monocytes, the transcriptome profile showed 87 upregulated and 12 downregulated DEGs, whereas in NK cells of AIH PBMCs, the profile showed 101 upregulated and 15 downregulated DEGs.	After GO functional enrichment analysis of the DEGs, cells with similar expression patterns were grouped using the UMAP method. The GEO database was constructed using the obtained data.	AIH is an immunological condition characterized by autoantibodies, hypergammaglobulinemia, and persistent active hepatitis. Understanding the molecular mechanisms in PBMC-derived monocyte and NK cell clusters could aid AIH therapy.	2024	18
3	Diabetic kidney disease (DKD)	The researchers used CellMarker and CellPhoneDB to analyze single-cell RNA sequencing data in publicly available datasets to identify cell types and interaction networks associated with DKD.	Six DKD-related datasets were analyzed using scRNA-seq and then normalized, after which 10 genes with the highest variation were determined. DEGs of each cell subtype were detected using the Find All Markers method.	The study identified 356 DEGs as implicated transcriptomes, with 178 upregulated and 178 downregulated genes.	The regulatory relationship between genes and traits associated with DKD was evaluated using WGCNA analysis, KEGG and GO databases, Metascape and GEO databases, and other relevant information.	RNA-seq analysis revealed 356 unique DEGs in the kidney, platelet alpha granule, urogenital system, and glycosaminoglycan-binding pathways, with three immune-related genes used as DKD treatment targets and diagnostic markers.	2023	19
4	Autoimmune diseases (psoriasis and vitiligo)	This study investigated the aberrant ferroptosis- and pyroptosis-related gene patterns in autoimmune diseases such as vitiligo and psoriasis using scRNA-seq datasets and bulk RNA sequencing.	The Genome Sequence Archive (GSA) provided scRNA-seq datasets for analysis, scoring target cells based on gene expression levels in signature gene lists.	Certain pro-ferroptosis genes, including *ACSL4*, *SAT1*, *RPL8*, and *CS*, showed increased expression in the transcriptome profile.	Gene expression analysis was performed using DESeq2, sequencing was performed using Illumina HiSeq™ 2000, and scRNA-seq datasets for lesional and non-lesional skin biopsies from patients with AD were accessed through the GEO database.	The study revealed that ferroptosis and pyroptosis disrupt vitiligo and psoriasis, providing new insights into their role in the scRNA-seq-based pathophysiology of autoimmune diseases.	2022	20

(Cont'd...)

Table 1. ***(Continued)***

S. No.	Condition/ disease	Study pattern	Methodology adopted	Transcriptome profile	RNA sequence analysis and database	Findings	Year of study	References
5	Breast cancer	Using RNA-seq and trajectory analysis, the researchers identified CD44+/ALDH2+/ ALDH6A1+ breast cancer stem cells (BCSCs) and verified their pluripotency.	This study involved five female patients with breast cancer, including triple-negative and non-TNBC types, and performed scRNA-seq analysis of primary tumors and matching lymph nodes.	Overall, 10 matching axillary lymphatic nodes and 96,796 individual cells from 5 primary tumors were used to construct UMAP maps. The levels of expression were represented using heatmaps.	The accession code GSE180286 was used to store the scRNA-seq data in the GEO database.	The study revealed transcriptome profiles, established the evolutionary history of carcinogenesis, described intra- and inter-tumor heterogeneity, and identified BCSCs.	2021	21
6	Systemic lupus erythematosus (SLE)	Researchers analyzed lncRNA profiles and dysregulation in the peripheral blood mononuclear cells of patients with SLE using RNA-seq of PBMCs and other published transcriptome datasets.	The study used 10 μg of total RNA. Cutadapt was used to remove adaptors and low-quality bases, and transcriptome data were retrieved from two other studies for RNA-seq data processing.	The transcriptome profiles of patients with SLE were significantly dysregulated, with >95% of coexpression combinations showing positive correlations, suggesting underlying transcription-promoting effects of lncRNAs.	Ten SLE samples were selected at random and five controls were used in the investigation. The NCBI GEO database contained the RNA-seq data.	The lncRNA pattern of patients with SLE, influenced by the Th cell differentiation-associated lncRNA AC007278.2, may regulate inflammatory response genes, improving the understanding of the role of lncRNAsin SLE etiology and development.	2021	22
7	Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)or COVID-19	Whole-transcriptome sequencing of the peripheral blood samples of 14 patients with COVID-19 and four healthy donors revealed four modules in the protein-protein interaction network of upregulated and downregulated DE mRNAs.	Gene sequencing libraries were created using TruSeq Stranded RNA Total Library Prep Kit, TRIzol reagent, fastp software, miRdeep2, FPKM, miRanda, and FPKM to evaluate the accuracy of the raw data.	The transcriptome profile revealed 25,482 DE mRNAs, with 4142 upregulated and 21,340 downregulated mRNAs and 9 upregulated and 14 downregulated miRNAs, with a fold change of 2.0 and $P < 0.05$.	This study used the GenCLiP 3, GeneCards, and Online Mendelian Inheritance in Man (OMIM) databases to identify inflammation-related targets, merging increased levels of DE mRNAs and inflammation targets, resulting in an elevated expression of 149 DE mRNAs for COVID-19.	Whole-transcriptome sequencing was used to identify mRNAs, lncRNAs, and miRNAs in the peripheral blood of healthy donors and patients with COVID-19 and analyze the "cytokine storm" mechanism.	2021	23
8	Cervical cancer	The study identified 140 DEGs in cervical malignant tissues, normal tissues, and SiHa cells after transcriptome sequencing, irrespective of HPV16 E6/E7 deletion.	The study used bioinformatics to analyze 140 DEGs, revealing their primarily enriched content in the cell cycle and DNA repair, with 20 genes examined and six verified through qRT–PCR.	The study analyzed 20 genes, including *CENPI*, *KIF14*, *RACGAP1*, *FOXM*, *EXO*, *KIF4A*, *PSRC*, *C19orf57*, *MCM6*, *TLX2*, *CIT*, *NDC80*, *MCM7*, GINS2, *ORC*, *NUSAP1*, and *UHRF*.	This study utilized the TF2DNA database.	It provided a novel understanding of how HPV oncogenes maintain a malignant state at the transcriptional level.	2019	24

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doi: 10.36922/itps.4449

Table 1. ***(Continued)***

S. No.	Condition/ disease	Study pattern	Methodology adopted	Transcriptome profile	RNA sequence analysis and database	Findings	Year of study	References
9	Pancreatic ductal adenocarcinoma (PDAC)	The surrounding benign pancreatic tissues and the matching tumor transcriptomes were examined in 10 patients who underwent PDAC surgery.	RNA-seq of all 20 samples was successfully performed using nucleotide distributions and high-quality parameters, with reading sequences being 2 × 76 bp long.	The transcriptome profile revealed 2736 DEGs with a false discovery rate of <0.05, including six microRNAs and 1554 upregulated and 1182 downregulated DEGs.	The TopHat2 alignment tool mapped 95.5% of the sequenced reads to the hg19 genome assembly, with the RefSeq database providing the number of components in each identified gene.	The study used RNA-seq technology to identify a higher number of DEGs than previous microarray-based expression profiles, which can be used for biomarker testing and therapy of pancreatic cancer.	2017	25
10	Breast cancer	The study examined the transcriptome expression patterns of HER2-positive, TNBC, and non-TNBC breast tumors using Illumina technology, resulting in 1.2 billion high-quality raw reads.	Ribosomal RNA was extracted from total RNA using RiboMinus Eukaryote Kits and assembled into transcripts using Cufflinks; PCA plots were constructed and clustered using Avadis NGS software.	mRNA sequencing identified 75,000 transcripts in samples, including GNB2L1, CANX, B2M, and SPARC, affecting cell motility, antiapoptosis effect, migration, and stress management, which are linked to breast cancer progression.	The TopHat program 1.1.4 was used to align the pair-end readings. The OMIM database was used.	The mRNA sequencing–centered approach can provide a more precise deconvolution of the transcriptional and post-transcriptional aspects of breast cancer.	2012	26

Abbreviations: RNA-seq: RNA sequencing; PBS: Phosphate-buffered saline; GO: Gene Ontology; GEO: Gene Expression Omnibus; KEGG: Kyoto Encyclopedia of Genes and Genomes; lncRNA: Long non-coding RNA; qRT–PCR: Quantitative reverse transcription–polymerase chain reaction; PCA: Principal component analysis.

Table 2. Recent studies on transcriptomic RNA-seq in RA

S. No.	Purpose	Study pattern	Sample type	Methodology	Justification	Year of study	References
1	This study aimed to identify proteomic, transcriptomic, and cellular markers that indicate abatacept resistance in patients with RA.	Varying responses to abatacept in patients with RA are attributed to unknown molecular pathways. The study clarified the low effectiveness of abatacept in RA using transcriptomic RNA-seq.	Blood samples	A study involving 22 patients with RA using abatacept measured gene expression, plasma protein, and surface molecule levels using flow cytometry and RNA sequencing, with seven responders and 50 non-responders.	The researchers found that distinct monocyte-derived transcriptome features before treatment account for the differences in abatacept effectiveness. The information was obtained through transcriptomic RNA-seq.	2024	46
2	The aim of the project was to use transcriptomic RNA-seq to identify genes and pathways related to RA.	Gene expression data from 202 patients with arthritis were used to create a synovium gene expression predicting model, followed by transcriptome-wide association analysis using FUSION software and RNA-seq.	Synovium	Samples from 245 patients who underwent knee replacement surgery were subjected to transcriptomic sequencing, genotyping, RNA extraction, and RNA-seq, with the human reference genome (hg19) mapped to the findings.	Transcriptomic RNA-seq analysis using relevant synovial tissues revealed genes previously linked to RA, providing novel information on the fundamental genetic composition of RA.	2024	47
3	This study aimed to use transcriptomic RNA-seq to discover discrete populations of macrophages and their distinguishing characteristics in the RA synovium to treat RA.	In synovial cells from patients with RA, single-cell RNA sequencing or scRNA-seq was used to identify gene fingerprints and subsets of cells.	Synovium	The study visualized macrophage spatial distribution using transcriptomic and scRNA-seq data, flow cytometry, immunofluorescence, and transcription factor analysis to study the macrophage polarization markers CD86 and CD206.	ScRNA-seq identified three macrophage cell clusters in RA synovial macrophages, revealing distinct polarized states and molecular markers, helping in the development of a unique therapeutic strategy.	2023	48
4	The aim of the experiment was to discover novel targets for therapy and provide fresh insights into the peripheral blood immunological processes of RA using transcriptomic scRNA-seq.	The study used scRNA-seq data from four RA samples and single-cell transcriptomic data from healthy control samples to understand RA development mechanisms, identify therapeutic targets, and develop disease settings.	Peripheral blood samples	Single-cell 3′-gene level libraries were generated from peripheral blood samples and processed using Cell Ranger software, and DEGs were identified using the Find Markers function of the Seurat package.	This study described the present status of the immune system and cell communication in the peripheral blood of patients with RA, including the gene expression patterns, PBMC abundance, and alterations in signaling pathways. Furthermore, it discovered a number of important cell subpopulations and particular genes that aided in the discovery of novel therapeutic targets.	2023	49

(Cont'd...)

Table 2. ***(Continued)***

S. No.	Purpose	Study pattern	Sample type	Methodology	Justification	Year of study	References
5	Transcriptomic RNA-seq was used in this study to address RA remission mechanisms, provide predictive biomarkers, and gain a deeper understanding of the role played by distinct fibroblast populations in the RA process.	To identify HBEGF+ fibroblasts and ascertain the number of HBEGF+ fibroblasts in various joint conditions (health, K/BxN serum transfer arthritis (STA), and STA remission), the study used two single-cell RNA sequencing datasets of mouse synovial cells.	Synovium	Thirteen datasets, including RNA-seq and single-cell transcriptomics, were used to analyze synovial tissue in 102 patients with arthritis, comparing gene expression between HBEGF+ and HBEGF− fibroblasts.	Fibroblasts in the synovial fluid are believed to play a crucial role in controlling joint homeostasis in RA. Using transcriptomic RNA-seq, the study found that HBEGF+ fibroblasts contribute to RA remission and that HBEGF may be a novel biomarker for predicting RA progression.	2022	50
6	To ascertain whether gene–gene interactions in a network analysis of synovial samples obtained during the early phases of RA using RNA-seq could contribute to our understanding of the pathophysiology of RA and improve treatment response in prediction models.	The study was designed to collect 10,537 experimentally confirmed gene–gene interactions using four carefully selected route libraries. After characterizing histologically defined pathotypes in early RA using synovial RNA-seq, we extracted particular gene–gene interaction networks and utilized these synovial-related gene–gene networks to predict the response to methotrexate-based disease-modifying antirheumatic drug (DMARD) therapy. Next, by statistically evaluating each network with robust linear regression models, the study revealed the differential interactions within each network.	Synovium	The study examined RNA-seq data from 94 patients with RA who started methotrexate-based csDMARD therapy after 6 months, evaluating gene–gene interactions through rigorous regression analysis.	To improve models for predicting treatment response in RA, the study included a unique, potent method known as transcripts micRNA-seq, which leverages physiologically significant gene–gene interactions through gene interaction networks.	2022	51
7	The aim of this study was to gain a complete understanding of the patterns of expression across the genome in synovial tissue samples from patients with RA to identify potential mechanisms regulating the onset and progression of RA.	RNA-seq was used to determine the transcriptomic patterns of synovial tissue specimens from nine patients with RA who were members of the East Asian community. All identified genes were examined using gene set enrichment analysis (GSEA), and DE-seq was used to identify differentially expressed genes (DEGs). Quantitative real-time PCR (qRT–PCR) was used to verify the most important hub genes.	Synovium	Samples of synovial tissue were collected from nine patients with RA. Total RNA was then extracted from the synovial tissue. Total RNA samples with RIN > 7.0 and 28S/18S ≥ 0.7 were subjected to RNA-seq. Then, libraries were constructed using TruSeq Stranded mRNA LT Sample Prep Kit. The Illumina HiSeq ×10 platform was used for assembling the libraries. The accuracy of the RNA-seq technique in detecting DEGs was evaluated, and the expression levels of the 10 identified hub genes were quantified using qRT–PCR.	This study identified and validated DEGs in synovial tissue samples from patients with RA using transcriptomic RNA-seq. It also highlighted the activity of a subset of chemokine genes and provided novel insights into the molecular mechanisms of RA pathogenesis. Finally, it identified potential targets for screening and treatment.	2022	52

(Cont'd...)

Table 2. ***(Continued)***

S. No.	Purpose	Study pattern	Sample type	Methodology	Justification	Year of study	References
8	The most prevalent internal alteration found in eukaryotic mRNAs is N6-methyladenosine (m6A), and it exerts a direct effect on the development and course of several diseases, particularly tumors. This study investigated the relationship between m6A methylation and RA using transcriptomic RNA-seq.	The aim of this study was to identify different amounts of m6A methylation in human RA using two high-throughput sequencing techniques.	Synovium	The study used the MH7A cell line to detect m6A methylation in RA, confirming the expression levels in MH7A cells and synovial tissues of AA rats.	A total of 206 genes with differentially regulated m6A methylation were detected using m6A-seq, of which 118 had substantially elevated m6A methylation and 88 had significantly decreased m6A methylation. In MH7A cells, the transcriptional mapping of m6A was performed, and a possible correlation between the RNA methylation alteration and RA-related genes was noted.	2021	53

Abbreviations: RNA-seq: RNA sequencing; RA: Rheumatoid arthritis.

poor quality. To identify the differentially expressed genes (DEGs) based on fold change and statistical significance criteria, techniques such as DEseq2 and limma-voom are used. Moreover, it performs DEG annotation using databases and bioinformatics tools, and DEG maps are used to identify pathways that are enriched in dysregulated genes according to predetermined criteria. Bar plots, bubble plots, and network diagrams are useful for showing the most important pathways.[33,34] Dysregulated pathways in RA include T-cell receptor signaling, altered B-cell receptor signaling, autoantibodies, and the Toll-like receptor. Type I IFN signaling is activated in RA, contributing to inflammation and joint destruction. The signal transducer and activator of transcription (STAT) regulates inflammation and the immune response, whereas nuclear factor-κB regulates gene expression. Abnormal expression of the Janus kinase–STAT pathway and the mitogen-activated protein kinase (MAPK) signal transduction pathway also contribute to RA pathogenesis.

3.4. Personalized medicine

The traditional treatment for RA involves medication, psychotherapy, and rehabilitation to maintain the locomotor system and reduce discomfort, inflammation, and osteoporosis. Weak opioids, muscle relaxants, and analgesics are used to manage pain, whereas strong opioids such as morphine are considered in certain cases. RNA-seq profiling can predict prognosis, treatment response, and disease progression. Synthetic disease-modifying antirheumatic medications include cyclosporine A, leflunomide, azathioprine, methotrexate (MTX), sulfasalazine (SSZ), and cyclophosphamide. RA is diverse, with MTX being the first-line treatment in 40 – 60% of cases.[35,36]

3.5. Immune dysregulation

Analyzing gene expression in immune cells and the synovium can assist in identifying dysregulated pathways and RA etiology using RNA sequencing. The expression patterns of cytokines and chemokines in the synovium and other tissues of patients with RA were also examined. Tumor necrosis factor-alpha, interleukin-6, and other chemokines can be used to identify the pathophysiology of RA. RNA-seq enables the profiling of autoantigens, immunological tolerance, and autoantibodies. Autoimmune problems are significant for patients with RA. Combining all omics data helps in the study of immune dysregulation (genomics and proteomics).[37-39]

4. Transcriptomic profiling of synovial tissue and fluid in patients with RA

The synovium, a thin tissue layer, aids in the function of diarthrodial joints by lining the fibrous capsule.[40] In healthy joints, it generates lubricin and hyaluronate for synovial fluid, supporting flexion and load-bearing without compromising bone and cartilage integrity. The inflammatory synovium in RA exhibits tissue hyperplasia, angiogenesis, and leukocyte infiltration, with fibroblasts playing a vital role in cartilage destruction, leukocyte influx and outflow, and bone damage.[41]

In RA, proinflammatory chemicals such as IL-6 and CCL5 are generated, synovium fibroblasts are constantly active, and matrix remodeling enzymes such

as matrix metalloproteinase (MMP)-9 and MMP-3 are secreted. Moreover, enhanced proliferation and reduced responsiveness to apoptotic signals are observed. Consequently, the synovium develops an aggressive nature, resulting in inflammation and hyperplasia, ultimately causing discomfort and loss of function. However, over the past 10 years, several fibroblast subpopulations have been identified through transcriptomic technologies and single-cell sequencing.

In each previously reported experiment, the synovial tissue was collected from limbs that had been amputated or from victims' limbs within 6 h of their death. Studies often end at one of two resolution levels: whole-tissue samples or individual cellular populations.[42,43] Two primary methods are commonly used for the isolation and investigation of synovial cells, also known as synovial fibroblasts: the explant outgrowth approach and the enzymatic digestion approach. The enzymatic digestion strategy focuses on breaking down the extracellular matrix and disrupting cell adhesion to this matrix to generate a suspension of synthetic cells.[43] However, a range of enzymes, concentrations, and digestion durations can be used to achieve this. Owing to the straightforward nature of the explant outgrowth approach, it is reported more frequently. When small segments of synovial tissue are cultivated, adherent cells can move out of the tissue and begin to proliferate.[42,44] After 7 days, the remaining tissues should be removed to cultivate cells. Although the explant outgrowth approach is easier to use than the enzymatic digestion approach, it has certain limitations. Favoring synovial populations that proliferate rapidly and are mobile enough to escape the tissue while ignoring other populations is risky. Furthermore, there exists a technique for cryopreserving the synovial tissue that minimizes batch effects and permits further digestion.[45] To avoid any contaminated proteins or DNA, the extracted RNA is purified. Reverse transcriptase is used to convert RNA into cDNA. Polymerase chain reaction (PCR) is used to amplify the fragmented cDNA library to provide sufficient material for sequencing. An Illumina NovaSeq or HiSeq sequencing platform is used to load the prepared library.

Low-quality reads and adaptor sequences are eliminated from the raw sequencing reads through processing. The abundance of each transcript is measured using bioinformatics techniques (gene expression analysis). Functional analysis of the DEGs is performed to gain insights into their biological functions and possible roles in the pathophysiology of RA. Researchers can use RNA-seq to obtain useful information on the gene expression profile of synovial cells in RA by following the techniques depicted in Figure 3. Using these data, we can better understand the mechanisms underlying RA and establish new diagnostic and treatment targets.

5. Identification of DEGs associated with RA pathogenesis

The most common type of autoimmune inflammatory arthritis in adults is RA. In addition to increasing the mortality rate, RA significantly reduces the quality of life in terms of health and impairs one's ability to perform everyday chores, including domestic and job-related responsibilities.[37,41] Five of every 1000 people have RA, which certainly causes serious damage to joints and disability.[54] Joint synovitis, which can appear as exudation, synovitis, or other diseases in the joint cavity, is a

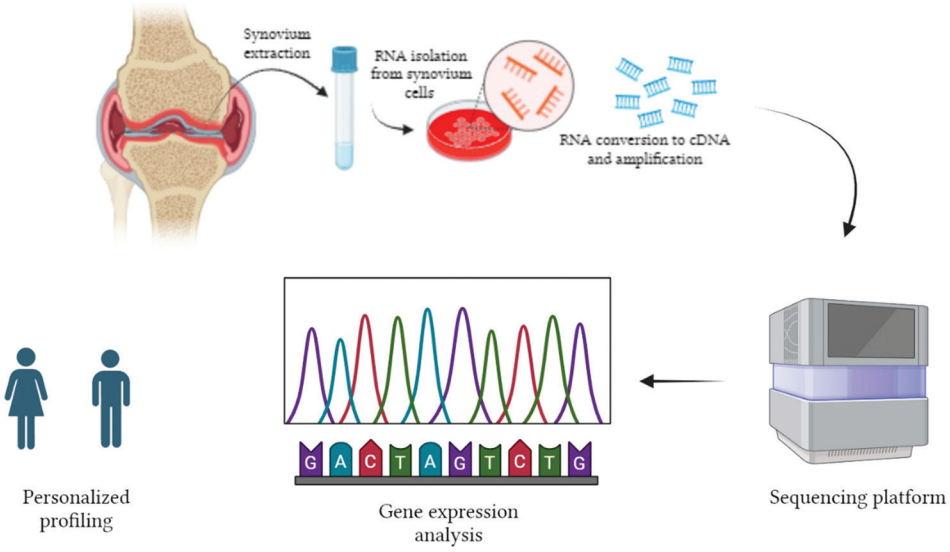

Figure 3. Transcriptomic gene expression profiling of synovial cells in rheumatoid arthritis

pathogenic feature. Finally, these disorders result in bone and cartilage dysfunction as well as stiff joints.[55,56] RA is a severe inflammatory autoimmune disease that is affected by both hereditary and environmental factors. In recent years, new genes and the close relationship between genetic and epigenetic processes have received considerable attention.[57] Serological testing (rheumatoid factor and anticyclic citrullinated peptide) and magnetic resonance imaging are the most frequently used techniques for the early detection and diagnosis of RA.[58] Nevertheless, these methods have limitations, and therefore, the precision and accuracy are poor.[59] X-rays and computed tomography can only identify lesions that are advanced in the stage; they cannot identify early damage. At present, there is no established therapy for RA, and its origin remains unknown. An increasing number of studies indicate that it is associated with genetics, immunological problems, endocrine variables, environment, and other factors, resulting in long-term inflammatory joint lesions. The recurrence and continuation of numerous complicated diseases or biological processes are intimately linked to the DNA methylation control of gene expression.[60] DNA methylation is crucial for preserving the expression profile unique to a given tissue, blocking the expression of foreign and viral genes, imprinting gene expression, and controlling chromatin stability.[61] DNA hypermethylation often prevents transcription factors from binding to the target area, which can restrict gene production; however, some studies showed that hypermethylation can also increase gene expression. The findings still require further investigation because of their inconsistency. Moreover, a plethora of research indicates that DNA methylation could play a role in the onset and progression of RA.[62] Gene expression microarrays have primarily been used to examine gene expression profiles, which provides a reasonably novel approach to exploring genes and numerous opportunities for drug-based molecular targeting applications. Numerous investigations of RA on methylation or expression microarray data profiling have been conducted in recent years, and hundreds of differentially methylated and differentially expressed genes (DMGs) have been identified.[63-67]

6. Regulatory networks and signaling pathways revealed by RNA-seq in RA

RA and osteoarthritis are joint diseases influenced by genetics, nutrition, and environmental factors. Transcriptomics and RNA sequencing can help differentiate between the two by examining gene expression.[51]

6.1. Role of RNA-seq in RA research

RNA-seq is a crucial technique in the study of RA, providing insights into the transcriptome and molecular mechanisms. It identifies genes that are upregulated or downregulated, allowing researchers to evaluate gene expression levels and identify coding and non-coding RNAs linked to RA. This information can be used for prognosis, therapy response monitoring, and disease diagnosis. RNA-seq also profiles gene expression in specific cell types, helping in targeted therapeutic development and drug discovery.[51,68,69]

6.2. Dysregulated gene expression in RA

RA is caused by dysregulated gene expression, resulting in inflammatory and autoimmune processes that damage joints. Dysregulated genes produce proinflammatory cytokines, causing synovial inflammation, cartilage degradation, and bone erosion. Autoantibodies and immune complexes cause tissue damage and synovial inflammation. Dysregulated transcription factors and abnormal signaling networks promote inflammation and immune cell activation. Anti-inflammatory mediators such as TIMPs and IL-10 balance proinflammatory and anti-inflammatory responses. Identifying these genes is vital for developing customized therapies to improve the prognosis of patients with RA.[4,70,71]

6.3. Regulatory networks in RA

Key transcription factors, including NF-κB, AP-1, and STAT3, exhibit dysregulated activity in RA, as shown by RNA-seq.[72,73] In the synovium of patients with RA, these factors are critical in regulating the production of proinflammatory cytokines, chemokines, and other inflammatory mediators. Gene dysregulation in RA is affected by epigenetic modifications, including chromatin structural alterations, DNA methylation, and histone acetylation.

Non-coding RNAs, including long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), have also been shown to play a role in RA pathophysiology. These RNAs possess the ability to target genes related to immunological responses, inflammation, and bone/cartilage homeostasis. In cRNAs interact with chromatin-modifying complexes or control the expression of target genes related to inflammatory signaling pathways and immunological responses. Moreover, circular RNAs act as microRNA sponges, absorbing miRNAs and altering their activity to indirectly regulate gene expression in RA-related pathways.[72,73]

6.4. Signaling pathways implicated in RA

Numerous signal transduction pathways affect the course of RA, and aberrant signals are frequently used as targets for developing medications. One of the most important members of the JAK family, the JAK-STAT pathway, controls apoptosis, cell differentiation, proliferation, inflammation,

and immunological function. The pathological foundation of RA is synovitis, and a variety of inflammatory responses have been observed in the synoviums of patients with RA (the soft tissue lining the interior of joints, tendon sheaths, and bursae is known as the synovium, also known as the synovial membrane or stratum synoviale). By suppressing JAK2 and STAT1, RA can be treated by activating T and B cells and preventing the production of cytokines.[74]

The pathogenic process of RA is intimately associated with the MAPK signaling system. The most significant member of the MAPK family, P38 MAPK, is activated in endogenous immune cells and can thicken the synovium, activate transcription factors, and significantly increase the production of inflammatory chemokines. It may also contribute to T-cell differentiation and prevent cell death. Angiogenesis, proliferation, metabolism, and cell survival are all regulated by the PI3K-AKT pathway, an intracellular mechanism that has been associated with the onset and progression of RA. The pathophysiology of RA is aided by the abnormal activation of this pathway, which increases the production of inflammatory chemicals and cytokines.[75]

In the PI3K/AKT/mTOR pathway, the mammalian target of rapamycin (mTOR) suppresses autophagy, encourages aberrant synovial cell proliferation, and is essential for osteoclast survival and differentiation, all of which exacerbate RA. SYK and BTK are two important molecules involved in B-cell receptor signaling, whose dysregulation has been linked to the etiology of RA. Phosphorylated SYK and BTK are expressed at higher levels in peripheral B cells in patients with RA, which is associated with the formation of autoantibodies. Regarding the treatment for autoimmune conditions such as RA, BTK is a desirable target.[75]

By controlling bone metabolism and synovial inflammation, the Wnt signaling pathway is essential in the pathophysiology of RA. Studies have demonstrated that the inhibition of NAV2 expression stops the development of RA and reverses phenotypes associated with inflammation, making it a prospective target for RA therapy.[74,75]

7. Integration of multiomics data to clarify the molecular mechanisms underlying RA

The term "multiomics data" describes the synthesis of several types of molecular data from different omics technologies, including transcriptomics, proteomics, metabolomics, and epigenomics.[76-78] Every omics technique provides a limited perspective on the various facets of biological systems. Proteomics investigates proteins and their alterations, metabolomics explores small molecule metabolites, transcriptomics examines gene expression levels, and epigenomics investigates epigenetic changes that control gene expression. Genomics is primarily concerned with DNA sequences and genetic variants. Combining these disparate omics datasets can enable researchers to obtain a more detailed knowledge of intricate biological processes and illnesses such as RA. A more comprehensive understanding of the molecular mechanisms underlying the pathophysiology of RA is possible through integration, which enables the investigation of interactions and correlations between genes, proteins, metabolites, and epigenetic variables.[79,80] RA is a mixed disease involving numerous biological processes, including inflammation dysregulation and tissue damage. Multiomics approaches allow an in-depth overview of the molecular landscape by continuously analyzing multiple biological layers of bio information such as transcriptomics and genomics. RA exhibits a diverse clinical presentation and response to treatment. Mutiomics data help identify molecular subtypes or clusters within patients, which helps in the treatment of particular individuals. Multiomics data help interconnect molecular pathways and networks and identify signaling pathways driving disease progression. Multiomics helps discover and develop new therapeutic agents for patients with RA. Researchers can repurpose current medications with established effects on relevant molecular pathways and prioritize prospective targets for therapeutic development by identifying molecular targets and pathways implicated in the disease etiology.[81,82]

8. Benefits, limitations, and significant advances of the technique

Several simple and complex diseases are poorly understood; hence, the analysis of their transcriptome is a vital method to investigate various diseases such as RA. Next-generation RNA-seq is a high-throughput method for the characterization of gene expression levels. Therefore, the use of RNA-seq in RA research has several benefits. This method can detect all RNA transcripts irrespective of the genes known or presently unknown, which provides a better view of the gene changes occurring in RA. RNA-seq provides precise and quantitative readouts of gene expression, therefore making it possible to capture differences in gene expression kinetics. It can also capture cases of alternative splicing, which results in the production of different proteins that are functionally different. RNA-seq can also identify unidentified RNA forms, for instance, ln cRNA and miRNA, which are responsible for the regulation of gene expressions. RNA-seq data obtained from RA samples can be combined with other omics data, including genomics and proteomics data, to further investigate the pathogenesis of RA.

Nevertheless, at the same time, there are some limitations to using RNA-seq. For instance, when performing large-

scale studies, RNA-seq can be relatively expensive. The processing of RNA-seq data is a delicate process and thus requires the use of specific visualization tools as well as extensive knowledge in the field of molecular and cell biology. Other limitations include specimen factors such as RNA degradation and sequencing errors. As mentioned earlier, RA is a heterogeneous disease and RNA-seq may not be an ideal method to detect upfold changes of genes in different cellularity of the joint.

RNA-seq has led to significant advances in our understanding of RA pathogenesis. It has provided novel genes of RA that are involved in immune cell activation, inflammation, and destruction of joints. RNA-seq has played some roles in the function of immune cells involved in RA, including Th17 cells, different T regulatory cells, and macrophages. It has provided tremendous insights into novel regulatory RNA molecules, including lncRNAs and miRNAs involved in the pathogenesis of RA. Some of the genes involved in immune cell signaling and inflammation have been proposed as therapeutic targets for RA using RNA-seq technology.

9. Conclusion

The use of next-generation RNA-seq has completely changed our knowledge of the intricate transcriptome landscape associated with RA. This review examined the use of RNA-seq in analyzing the pathophysiology of RA, ranging from defining dysregulated pathways and gene expression patterns to discovering novel biomarkers. Important discoveries include the identification of alternative splicing events, DEGs linked to RA pathophysiology, and the potential use of lncRNAs and circular RNAs as diagnostic tools. The complex regulatory networks and signaling pathways involved in RA have also been clarified using RNA-seq. Researchers can obtain a more comprehensive understanding of the molecular pathways causing RA by combining multiomics data. These findings are extremely promising for the development of tailored treatment approaches, improvement of diagnostic capabilities, and eventually, the discovery of novel therapeutic targets for individuals with RA. Prospective avenues for investigation may focus on overcoming constraints such as data analysis obstacles and integrating spatial transcriptomics data to comprehend cellular heterogeneity in the combined microenvironment. Certainly, additional research into the potential of RNA-seq will result in important discoveries in the field of RA research, providing better patient outcomes.

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REVIEW ARTICLE

Subanesthetic ketamine for reducing the harm of cocaine use disorder

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Abstract

Subanesthetic ketamine offers promising potential for reducing harm in individuals with cocaine use disorder (CUD). Research indicates that even a single dose can lessen cravings and decrease drug-seeking behaviors, though achieving long-term abstinence remains challenging. However, reduced cocaine consumption itself is a meaningful outcome. Ketamine's potential in reducing the harm of CUD is also supported by its mechanism of action in the dopaminergic system, as it counters cocaine's effect by interacting with dopamine receptors, stabilizing brain-derived neurotrophic factor levels, and modulating lateral habenula neuron bursting. In addition, concerns about ketamine's abuse potential are minimized when it is administered in a clinical setting under professional supervision. This is supported by its success as a treatment for depression, indicating that, with appropriate safeguards, ketamine could be a valuable pharmacological strategy for harm reduction in CUD. When developing ketamine as a CUD harm-reduction strategy, it is also important to account for sex differences, which may affect patients' sensitivity to ketamine and the potential for misuse. Although the promising effects of ketamine in treating depression support its use for CUD, most studies have focused on depression models, and additional research is needed to confirm safety and understand its specific mechanisms in CUD. Nonetheless, subanesthetic ketamine is a promising CUD intervention and should be further explored to provide an efficient and safe solution for patients in need. This narrative review mainly elucidates the ongoing research regarding ketamine's mechanisms of action, pharmacology, and clinical application potential in CUD.

Keywords: Cocaine use disorder; Ketamine; Dopamine; Nucleus accumbens; Ventral tegmental area; N-methyl-D-aspartate receptor; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; Brain-derived neurotrophic factor

1. Introduction

Cocaine, the second most used illicit drug worldwide, remains a significant public health concern.[1] According to the United Nations Office on Drugs and Crime, in 2020, the number of past-year cocaine users reached over 20 million worldwide.[2] Like other substance use disorders, cocaine use disorder (CUD) is a chronic condition represented

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by an inability to control drug seeking and intake despite damaging consequences.[3] The addictive property of cocaine is associated with its ability to interact with the mesolimbic pathway (Figure 1). This pathway includes the ventral tegmental area (VTA) innervating the ventral striatum, which includes nucleus accumbens (NAc), an area important for forming and maintaining addiction-related memory and contributes to relapse.[4] Most NAc neurons are medium spiny neurons (MSN), which are γ-aminobutyric acid (GABA)ergic neurons classified by the type of dopamine receptors they express. Although signaling pathways of these two types of neurons are highly complex, MSNs that express type 1 dopamine receptors (D1-MSN) are classically associated with rewards.[5-7] MSNs that express type 2 dopamine receptors (D2-MSN) can lead to decreased motivation and locomotion[8,9] (Figure 1).

Cocaine interacts with the mesolimbic pathway by remodeling glutamatergic synapses, especially those in D1-MSN,[10-12] creating "silent synapses," which are immature glutamatergic synapses that contain N-methyl-D-aspartate receptors (NMDAR) but not α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR).[13] NMDAR is a glutamate-gated calcium channel that activates when AMPAR-induced depolarization displaces a magnesium ion blocking it (Figure 2). Lacking AMPARs, these "silent synapses" cannot be activated and thus are not considered mature.[14,15] During cocaine withdrawal, these synapses mature quickly by recruiting AMPARs, leading to depolarization, NMDAR opening, calcium ion release, and eventually long-term potentiation, thus promoting addiction.[12]

Despite decades of research, most treatments for CUD rely on psychosocial interventions as there are currently no Food and Drug Administration (FDA)-approved pharmacological treatments.[1] However, ketamine, a non-competitive NMDAR antagonist, has gained attention as a promising pharmacological treatment for CUD. Ketamine is a racemic mixture of two enantiomers, S and R ketamine, with the S enantiomer (esketamine) being more potent. While ketamine has been used as a general anesthetic due to its dissociative effects, a subanesthetic dose of esketamine has been approved to treat depression[16] Although ketamine has a history of misuse as a recreational drug,[17,18] no overdose or fatality has been reported when it is used in a therapeutic setting.[18,19] Recently, due to ketamine's antagonistic effect and the association between depression and substance use disorders, there has been a lot of interest in exploring its therapeutic potential in treating various types of addictions.[20]

In this narrative review, we summarize the evidence presented in both pre-clinical and clinical studies illustrating ketamine's effect in reducing cue-induced

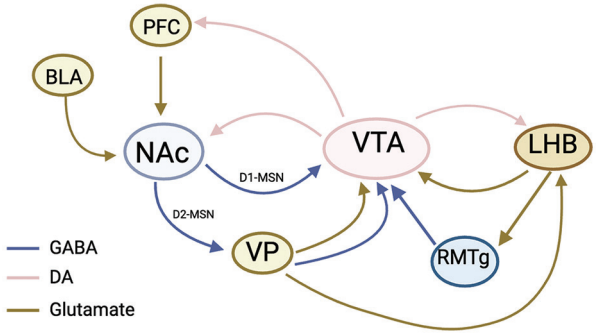

Figure 1. The brain circuits involved in cocaine use disorder prominently feature the ventral tegmental area (VTA), which plays a critical role in motivation, sending dopaminergic projections to the prefrontal cortex (PFC), nucleus accumbens (NAc), and lateral habenula (LHB). The NAc, a vital hub in this network, receives glutamatergic inputs from areas such as the PFC and basolateral amygdala (BLA). Subsequently, the NAc can project GABAergic signals directly to the VTA or indirectly through the ventral pallidum (VP). The direct pathway enhances cocaine-related conditioning, whereas the indirect pathway serves to inhibit it. Recent research suggests that both pathways can influence either reward or aversion, depending on the stimulation patterns.[6] In addition, the LHB sends direct excitatory projections to the VTA but can also inhibit VTA dopaminergic neurons by activating the rostromedial tegmental nucleus, which in turn delivers GABAergic signals to the VTA.[64,122]
Abbreviations: DA: Dopamine; GABA: γ-aminobutyric acid; MSN: Medium spiny neuron.

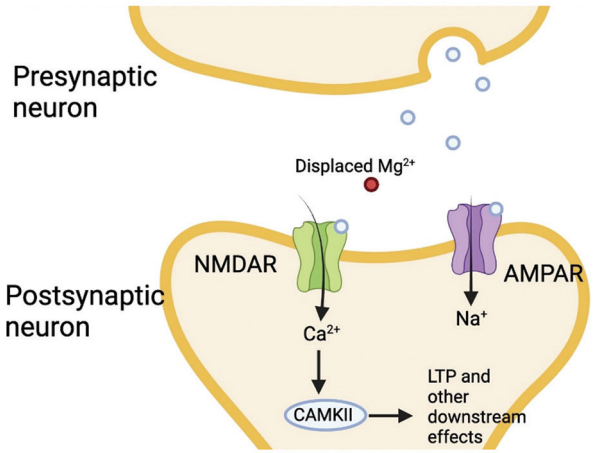

Figure 2. The intricate workings of NMDAR and AMPAR on post-synaptic neurons. These two glutamate-gated ion channels, which allow sodium or calcium to flow through when glutamate binds, are a testament to the complexity of neuroscience. NMDAR, with its slower kinetic than AMPAR's due to magnesium ion blockage, is a fascinating subject of study. Its activation can lead to downstream effects such as long-term potentiation or depression through calcium ion activation of targets like CAMKII.[123]
Abbreviations: AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CAMKII: Calcium/calmodulin-dependent protein kinase II; LTP: Long-term potentiation; NMDAR: N-methyl-D-aspartate receptor.

craving and drug-seeking behaviors in cocaine users while acknowledging its potential for misuse. In addition, we review ketamine's potential mechanisms and sex-dependent differences in ketamine's therapeutic effect. Finally, we identify a barrier to developing interventions for cocaine abuse as the expectation of abstinence. Grounding the approval standards for pharmacological interventions on more realistic patient-centered outcomes[21] would allow for the repurposing of ketamine as an efficacious harm-reduction medication for CUD.

2. Pre-clinical studies on ketamine's effectiveness in reducing cocaine use

In pre-clinical settings, subanesthetic ketamine can decrease cue-induced drug-seeking behaviors and lead to changes in brain connectivity associated with increased executive control. Fitzpatrick and Morrow[22] conducted a study using a Pavlovian-conditioned approach to investigate the effect of subanesthetic ketamine in rats, focusing on two behaviors: sign-tracking and goal-tracking. Sign tracker rats, who attribute motivation incentives to reward cues, display increased drug-seeking behavior, whereas goal tracker rats do not model addictive behavior. Subanesthetic ketamine at 32 mg/kg dose decreased sign-tracking behavior in sign-trackers without affecting goal-tracking behavior in goal-trackers, with effects lasting up to 48 h post-administration.[22] This indicates that a subanesthetic dose of ketamine reduced reward-seeking behaviors in the rats that were most vulnerable to impaired executive function related to addiction. Similarly, a study on cocaine-exposed rhesus monkeys evaluated the effects of subanesthetic ketamine on functional brain connectivity and cocaine self-administration.[19] Ketamine treatment at 48 h before cocaine self-administration reduced the cocaine-induced decrease of functional brain connectivity between brain regions involved in decision-making and reward processing. A separate cohort of cocaine-exposed rhesus monkeys exhibited reduced drug- and cue-induced reinstatement following subanesthetic ketamine.[19] These findings demonstrate ketamine's ability to decrease drug intake by modulating the interaction between different brain areas.

3. Clinical studies on ketamine's effectiveness in reducing cocaine use

Although clinical studies provide relevant models of substance abuse behaviors, their findings may not always translate directly to human subjects. Nonetheless, many clinical research results were consistent with that of the pre-clinical studies. In an observational study, Gao *et al.*[23] used artificial intelligence, validated by specialists, to identify ketamine as the best candidate for reducing the harm of CUD. They then assessed ketamine's therapeutic effects retrospectively using data from a de-identified patient database. Compared to cohorts who received other antidepressants or anesthesia medications, cohorts that received ketamine had a higher CUD remission rate.[23] Prospective clinical studies also support subanesthetic ketamine's effectiveness in decreasing craving and drug-seeking behaviors. In a 2013 clinical trial, Dakwar *et al.*[24] evaluated the effect of subanesthetic ketamine in eight non-treatment-seeking cocaine-dependent participants. A single ketamine infusion significantly increased motivation to quit and reduced cue-induced craving, demonstrating ketamine's potential to motivate behavioral change. The same investigators later explored whether a single subanesthetic dose of ketamine could disrupt cocaine self-administration in a cohort of 20 cocaine-dependent individuals disinterested in abstinence.[25] After infusions of subanesthetic doses of ketamine, participants chose between immediate cocaine self-administration and delayed monetary rewards. Remarkably, a single ketamine dose also significantly decreased cocaine self-administration. A hallmark feature of substance use disorders is a persistent desire to use the drug such that it becomes the only goal of the affected individual's life.[1] The fact that a single subanesthetic dose of ketamine enabled non-treatment-seeking subjects to choose money over cocaine is a testament to ketamine's potential as a CUD harm-reduction therapy.

4. Abuse potential of ketamine

Due to its history of recreational misuse, ketamine as a treatment for CUD may raise concerns about abuse potential and adverse effects.[26] Ketamine use for recreational purposes has increased in recent years, as reflected by a significant rise in ketamine-related seizures from 2012 to 2019.[27] However, ketamine differs from substances such as cocaine in that ketamine does not modulate synaptic plasticity in the same way.[28] Both clinical and pre-clinical studies suggest low abuse potential at subanesthetic doses of ketamine. For instance, a study found that daily ketamine doses of 2.5 and 5 mg/kg over 6 days failed to evoke a conditioned place preference in rats.[29] In addition, although the S enantiomer of ketamine has a more substantial behavioral effect and is responsible for ketamine's abuse potential,[30,31] most clinical trials use a racemic mixture of ketamine to improve CUD outcomes. Because substance abuse is greatly context-dependent, when subanesthetic ketamine is administered in professionally controlled settings, ketamine's abuse risk is minimized by effective monitoring and administration

in medical contexts.[19,26] Dakwar *et al.*[24] reported that high and low subanesthetic doses of ketamine could be safely given under controlled conditions to participants with CUD who do not engage in ketamine misuse. In addition, the repurposing of subanesthetic ketamine for treatment-resistant depression (TRD) illustrates the feasibility of employing ketamine as a CUD intervention. The nasal spray Spravato, composed of the S-ketamine enantiomer, was approved by the FDA as a treatment for TRD in 2019.[32] Spravato is accessible through a controlled distribution program in the United States and Canada, which requires direct supervision and monitoring by a certified health-care professional for at least 2 h post-dose.[26] The same distribution model can be utilized for the treatment of CUD with subanesthetic ketamine to ensure cocaine-dependent individuals can safely receive medical care.

Although research shows that administering subanesthetic doses of ketamine should be safe in clinical settings, ketamine misuse can still cause unpleasant side effects and pose health risks.[33] Ten to twenty percent of ketamine users experience the emergence phenomenon, which is a collection of symptoms including hallucinations, delirium, euphoria, and paucity of speech.[33] Ketamine can also cause changes in heart rate and blood pressure, posing risks for users with a history of myocardial infarction or hypertension.[33] Nonetheless, the side effects of ketamine are both a curse and a blessing when considering its potential as a pharmacotherapy. Subanesthetic doses of ketamine might also cause a range of psychoactive effects, some of which might appear like "spiritual" experiences. They might assist in individuals' transition from one lifestyle to another guided by ethical values.[34] Dakwar *et al.*[35] performed two more clinical trials to investigate whether these effects contribute to the decrease in cocaine-seeking behavior. In 2014, the group recruited eight participants with CUD. The exposure group received two ketamine infusions of different doses separated by 48 h, and another group received the positive control lorazepam.[35] In a 2019 follow-up study, the researchers examined 20 participants over three 6-day hospitalization sessions. Participants received either ketamine or positive control during the second and third hospitalization sessions. Both studies found that mystical effects mediated ketamine's effectiveness in reducing cocaine-seeking behavior, though the 2019 study also noted that these effects might reflect individual sensitivity to ketamine rather than its therapeutic impact alone.[36] Thus, some of ketamine's psychiatric side effects of ketamine might even be the underlying mechanism of its ability to improve CUD remission.

5. Potential mechanisms behind ketamine's harm-reduction effect

Despite the clinical and pre-clinical evidence pointing to ketamine's potential in care for CUD, the mechanisms behind its therapeutic effects remain contentious. One possible mechanism might be implicated in ketamine's effectiveness in treating depression, as clinical studies have been investigating anti-depressants' potential in treating substance use disorder.[23,37] The co-occurrence of major depressive disorder (MDD) and substance abuse is common,[38,39] and cocaine use itself can also induce depression during periods of heavy drug use.[40,41]

In addition, studies find that both cocaine-induced depression and independent MDD are associated with substance use relapse in both rats and humans.[41,42] Thus, this bidirectional relationship – where cocaine can trigger depression, and depression can hinder remission from use – underscores the importance of addressing both conditions simultaneously. Given that depression, CUD, and ketamine all modulate overlapping neurological pathways, ketamine may be particularly well-suited for treating CUD by targeting these shared mechanisms.

5.1. Ketamine directly modulates the dopaminergic system

One possible mechanism is that ketamine modulates the monoamine systems that are involved in both cocaine addiction and depression. Cocaine withdrawal often triggers depressive symptoms, which, unlike MDD, temporarily improve with cocaine use and respond poorly to selective serotonin reuptake inhibitors.[43] The association between cocaine and depression is due to cocaine's mechanism of action. Cocaine works by binding to the dopamine transporters (DAT) responsible for the reuptake of dopamine and by upregulating D1 dopamine receptors (D1Rs), which have stimulatory effects and are often involved in compulsive drug intake.[44,45] Both actions can lead to an increase in dopamine signaling.[46]

When cocaine use stops, there is an acute decrease in striatal dopamine levels[47] and a reduction of dopamine receptor 2 (D2R) signaling in the ventral striatum due to reduced D2R sensitivity.[48] Such reduction is also seen in independent depressive disorder, which is associated with a decrease in several monoamines, including serotonin and dopamine.[49] In addition, long-term cocaine users will develop tolerance, leading to increased DAT density and reduced dopaminergic (DAergic) signaling,[48,50] thereby reinforcing cocaine use.

Ketamine, which also affects the DAergic system, may potentially reverse cocaine's effect. Studies found that

ketamine increases the firing rate of DAergic neurons in the VTA for up to 24 h post-injection,[51] potentially due to its ability to activate D1R[52,53] by inhibiting several microRNAs in the medial prefrontal cortex (mPFC).[54,55] This can correct the low dopamine level and depressive symptoms seen during cocaine withdrawal, as activating D1R contributes to ketamine's antidepressant effect by promoting spinogenesis and dendritic plasticity.[55] Consistent with this finding, ketamine-induced behavior sensitization is abolished by a D2R antagonist, suggesting ketamine may activate D2R as well.[56] This supports ketamine's treatment potential for CUD, as the activation of D2-MSN in NAc suppresses cocaine self-administration.[8,57]

Interestingly, another study found that neither ketamine nor its metabolite showed any antagonist/agonist effect on D1R or D2R. Instead, ketamine's effect on dopamine is mediated by its effect on AMPA and NMDA receptors on VTA DAergic neurons.[9,58] It has been shown that acute ketamine administration increases DAergic neuron firing by amplifying AMPA transmission, whereas AMPAR antagonists abolish this effect.[59,60] Consistently, chronic ketamine administration led to an increase in AMPAR but not NMDAR density, pointing to ketamine's direct impact on AMPARs.[60]

Despite ketamine's therapeutic potential, there is still concern about its abuse risk, especially as it activates D1R, increases DAergic neuron activity, and inhibits DAT similarly to cocaine.[61-63] However, ketamine does not induce the same drug-evoked synaptic plasticity, especially at a subanesthetic dose. This is because synaptic plasticity generally requires NMDAR activation for excitatory synapse potentiation onto VTA DAergic neurons, while ketamine blocks NMDARs. In addition, ketamine's fast off-kinetic profile prevents prolonged dopamine increases that would otherwise promote synaptic plasticity.[28] Thus, despite its similarity with other addictive substances, ketamine's mechanism of action suggests a lower abuse potential. Nonetheless, more research needs to be done to elucidate the best method to take advantage of ketamine's therapeutic effect in clinical settings.

5.2. Ketamine modulates lateral habenula (LHb) neuron bursting

Another mechanism through which ketamine can alleviate depressive symptoms is by blocking neuronal bursting activity in the LHb. The LHb is associated with negative reward processing, as it stimulates the rostromedial tegmental nucleus, which inhibits VTA DAergic neurons (Figure 1).[64] Based on their activity, LHb neurons could be categorized as: silent, tonic-firing, and burst-firing.[65] Several studies found that, in animal models of depression, potassium channels in astrocytes near LHb are upregulated, leading to hyperpolarization. This change in membrane potential can lead to LHb bursting, a pattern dependent on the activation of NMDAR.[66,67] This type of bursting increases the likelihood that action potentials will reach the downstream synaptic target, thus enhancing the inhibitory effect of LHb on midbrain DAergic neurons.[68]

In a mouse model, optogenetically inducing this bursting pattern in LHb neurons can lead to depressive-like symptoms whereas blocking NMDAR rescued depressive-like symptoms.[64] Consistent with these findings, Ma *et al.*[69] reported that systemic ketamine injection significantly reduced bursting neurons in mice brain slices just 1-h post-injection, with effects lasting up to 24 h. Although these studies are mostly done using depression models, cocaine's ability to induce depressive symptoms through similar pathways underscores ketamine's potential in treating CUD. A study found that cocaine administration increased the phosphorylation at S845 of GluA1, a subunit of AMPAR in the LHb.[70] Phosphorylation at this position can increase AMPAR insertion on the cell surface and the probability of channel opening, thus increasing the chance of NMDAR activation.[71] Interestingly, although blocking AMPAR did not decrease burst firing in LHb neurons, increased AMPAR current or administration of AMPA increased burst frequency.[66] These findings suggest that cocaine may cause depressive symptoms by trafficking AMPARs to cell membranes, thus increasing LHb neuron bursting activity. Ketamine can then target this process by blocking the downstream NMDAR to stop the bursting of LHb neurons, thus assisting in CUD remission. These studies demonstrate that ketamine may reverse the depressive symptoms associated with CUD by preventing LHb bursting.

5.3. Ketamine modulates brain-derived neurotrophic factor (BDNF) levels

A third mechanism by which ketamine may aid in CUD treatment is through its modulation of the BDNF level, the most abundant neurotrophic factor in the nervous system. By binding to its receptor tropomyosin kinase B (TrkB), BDNF can modulate addiction-related neuronal plasticity.[72] Cocaine's effect on BDNF varies depending on the stage of the addiction cycle.[73,74] Research indicates that serum or plasma BDNF levels increase with acute cocaine use and during periods of cocaine abstinence but decrease with prolonged cocaine use.[75-79] This is consistent with the co-occurrence between cocaine use and depression since a decrease in BDNF is also associated with depression.[80] Interestingly, during withdrawal, a high level of BDNF is associated with cocaine craving, and cocaine-induced depression has a higher BDNF level compared to primary

MDD.[77,81,82] Thus, instead of consistently up- or down-regulating BDNF, cocaine appears to cause fluctuation in BDNF levels that contribute to the addiction.[74]

Consistent with ketamine's interaction with the mesolimbic system, ketamine can also modulate BDNF, which, along with TrkB, is expressed in the VTA-NAc circuit.[83] Several studies found that ketamine can increase BDNF expression in various brain areas, an effect that can last up to 30 days after a single infusion of ketamine.[84,85] In clinical studies, higher plasma BDNF level is associated with the anti-depressant effect of ketamine in patients.[86,87] Other studies also directly demonstrated BDNF's effectiveness in reducing cocaine consumption, as pre-clinical research shows that a single injection of BDNF to the dorsomedial prefrontal cortex and NAc can decrease cocaine-seeking behavior.[88] Notably, such effect is specific to cocaine and no other natural awards such as sucrose solution.[87,88] Together, this evidence suggests that ketamine might facilitate CUD treatment by stabilizing the BNDF level. Nonetheless, studies that specifically explore this relationship should be conducted.

One limitation of the clinical studies mentioned above is that most measured plasma BDNF as a proxy for brain BDNF levels. This is because BDNF crosses the blood–brain barrier easily, and there is a high correlation between plasma and cerebral spinal fluid concentration in BDNF.76 However, BDNF's effects vary across brain regions. For example, increased VTA BDNF expression enhances cocaine seeking, but injection into the dorsomedial prefrontal cortex attenuates CUD relapse.[89,90] Thus, while plasma or serum BDNF is the best choice for clinical studies, pre-clinical studies can further elucidate the brain area where BDNF acts to facilitate ketamine's therapeutic effect on CUD.

6. Sex differences in response to ketamine

While plenty of evidence illustrates ketamine's potential effectiveness in treating CUD, pre-clinical studies also suggest that there might be a difference in ketamine's harm-reduction effect in males versus females. For instance, ketamine rescued social isolation-induced decreases in mPFC spine density in male but not female rats.[91] In addition, continuous administration of a subanesthetic dose of ketamine resulted in antidepressant effects in male rats but anxiety- and depressive-like behavior in female rats.[92]

6.1. Which hormones are responsible for these sex-dependent differences?

Although evidence points to a sex-dependent response to ketamine, studies differ on which sex hormone causes this difference. Multiple pre-clinical studies showed that females are more sensitive to ketamine at a lower dose,[93] and its effect is estrogen-dependent but not testosterone-dependent.[94] For instance, in response to ketamine, there is a significant increase in BDNF levels in female depressed rats compared to male ones.[95] Specifically, proestrus, but not diestrus, female C57BL/6 mice exhibited enhanced sensitivity to a very low dose of ketamine (1.5 mg/kg), possibly due to estradiol's effect on synaptic plasticity.[96] These studies seem to point to estrogen as the reason behind females' heightened sensitivity to ketamine.

However, several other studies that investigated the pharmacokinetics of ketamine found the male hormones to be responsible for the sex-dependent differences. Highland *et al.*[97] found that, compared to male rats, female CD-1 rats have a higher plasma level of a ketamine metabolite at 10 and 30 min after ketamine injection. This metabolite, hydroxynorketamine, is responsible for the antidepressant effect of ketamine. On the other hand, male rats have a higher plasma level of unmetabolized ketamine. While ovariectomy did not eliminate the difference between female and male ketamine metabolism, orchidectomy did, suggesting that testosterone may play a role in ketamine metabolism.[97] This is inconsistent with another study by Saland *et al.*,94 which found that female Sprague-Dawley rats have higher ketamine plasma levels 30 min post-injection. Yet, since Saland *et al.*[94] did not find a difference between proestrus and postestrus rats, both studies agreed that female hormones do not play a role in the sex-dependent difference in ketamine pharmacokinetics. In addition, studies found that ketamine might exert its effect partially through interacting with mu-opioid receptors, and this effect was only significant in male rats.[98,99] This opioid-dependent effect is reversed by orchiectomy, suggesting that testosterone plays a role in the ketamine effect.[98] This is consistent with a study that showed concurrently administering naltrexone, a mu-opioid receptor antagonist, with ketamine in a female human subject did not block ketamine effects.[100] Thus, these studies suggest that testosterone could either be driving the observed sex differences in ketamine sensitivity or that males are more sensitive to ketamine, directly opposing the studies mentioned in the previous paragraph.

6.2. Sex- and age-dependent differences in ketamine's abuse potential

In addition to its therapeutic effect, ketamine's abuse potential may also be sex-dependent. A study found that female rats exhibit higher locomotor sensitization to repeated antidepressant doses of ketamine,[33] with this effect occurring at a lower dose in female rats than in male rats.[101] Locomotor sensitization is the increased

behavioral response in rodents to repeated exposure to psychostimulant drugs, and it shares features with addiction in humans.[102,103] Thus, these studies suggest that females are at a higher risk of ketamine abuse compared to males.

However, studies have also shown that, at a higher dose (20 mg/kg), ketamine administration in juvenile male rats increased the preference for cocaine during adulthood. This effect was not observed in female rats at any age, suggesting that males might be at a higher risk for ketamine-related drug abuse potential.[104] Another study investigating ketamine's abuse potential at 80 mg/kg found female Sprague-Dawley rats across all ages produced high locomotor activity, whereas only preweaning male Sprague-Dawley rats showed a similar response, with adult and adolescent males displaying less activity.[105]

One reason for the discrepancy in research results might be that different strains of rats may respond differently to ketamine.[106] Taken together, these results suggest that both age and sex need to be considered when exploring ketamine's potential side effects.

6.3. Implication for developing ketamine as CUD harm-reduction strategy

The pre-clinical research results mentioned above highlight the importance of continuing to explore the sex-dependent effect of ketamine. This is especially important since cocaine addiction is also sex-dependent, with females being more likely to progress through the addiction cycle, and cocaine-seeking behavior correlates with female sex hormone levels.[107,108] However, it is also important to recognize that the biological differences between humans and rats may render pre-clinical research results less beneficial when developing ketamine as a treatment.

Clinical research did not seem to find that humans exhibit sex-dependent responses to ketamine. One clinical study that investigated ketamine's antidepressant effect found that, although there was no significant difference in ketamine's treatment response in males versus females, females reported more headache and nausea as ketamine's side effect. However, the difference between the male versus female side effects was not significant.[109] In addition, a 2022 systematic review of 27 clinical studies published from 2012 to 2020 found no significant sex difference in response to ketamine treatment.[110]

The discrepancy between the conclusions of the rat and human studies might be due to the size limitation in most clinical trials. While the biological differences between rats and humans should not be overlooked, there is a need for larger trial clinical studies exploring how sex hormones influence response to ketamine therapy in humans. In addition, since most studies of ketamine explore its antidepressant effect, it would be beneficial to specifically study female versus male responses to ketamine's therapeutic effect on CUD.

7. Abstinence or use reduction as primary goal of pharmaceutical intervention of CUD

While evidence suggests that ketamine may reduce cocaine use, research on whether subanesthetic ketamine can lead to long-term abstinence generated mixed results. Dakwar *et al.*[24] measured cocaine use behavior a month after ketamine treatment and found that a mere two of the eight participants achieved urine-confirmed abstinence at follow-up. However, the same group performed a more extensive study with 55 patients who received a single ketamine infusion or midazolam during 5 days of hospitalization. Each participant also attended daily sessions of mindfulness-based relapse prevention (MBRP).[111] After the 1st week, the participants received 4 weekly sessions of MBRPs. Six months later, a follow-up interview is conducted through telephone to assess cocaine use. This study found that ketamine is associated with lower levels of cocaine use, craving, and time to relapse. A significantly greater proportion of people remained abstinent during the 6-month interview. This study shows the potential of using ketamine as a pharmacotherapy in conjunction with behavioral therapy to treat CUD.[111] However, future studies with larger, more diverse samples and objective evaluations of cocaine use at 6 months are needed to assess the effectiveness of subanesthetic ketamine better.

Although abstinence is often the primary goal in CUD interventions, recent research confirms that reducing cocaine use can be considered a viable treatment outcome for cocaine-dependent individuals.[112] For instance, Amin-Esmaeili *et al.*[113] analyzed data from 13 randomized controlled clinical trials on pharmacological interventions for cocaine or methamphetamine use disorder. They found that individuals who reduced their use frequency from high to low experienced improvements in drug-related symptoms, cravings, and comorbid depression scores. Notably, a significant portion of these individuals tested negative for drugs in urine tests 1 month later, indicating that reduced use can improve quality of life and support further reductions.

Similarly, Roos *et al.*[114] used data from seven randomized controlled trials for CUD to evaluate the clinical utility of reduction in cocaine use frequency as a non-abstinence endpoint. The investigators separated participants into three levels at baseline and end-of-treatment (EOT): Abstinence, low frequency, and high frequency. In general,

individuals who reached the low-frequency level at EOT had similar outcomes as those who reduced to abstinence and significantly better outcomes than those who remained at the high-frequency level. Specifically, at 12-month follow-up, participants with at least one level of reduction at EOT displayed fewer psychological problems, employment issues, and cocaine use days. This study demonstrates that cocaine use reduction can improve overall well-being in the long term. Considering these results, reduced use should become an acceptable treatment objective in CUD interventions.

Supporting this perspective, the Substance Abuse and Mental Health Service administration released an advisory in 2023 encouraging "low barrier care," which emphasizes harm reduction by setting substance use reduction – not abstinence – as a primary goal.[115] This is supported by the U.S. FDA's guidance stating that outcomes other than total abstinence are acceptable endpoints for cocaine addiction clinical trials.[116] While abstinence remains the ideal goal, participants with high use at baseline may deem abstinence as unattainable. The introduction of reduced use as a primary goal could motivate cocaine-dependent individuals to work gradually toward abstinence. Given its potential to reduce cocaine use and the clinical validity of reduction as an outcome, subanesthetic ketamine should be regarded as a promising pharmacological option for CUD.

8. Ketamine versus other pharmacotherapy as CUD harm-reduction strategy

At present, the standard treatment for CUD primarily involves psychosocial treatments, which include cognitive behavioral therapy, motivational interviewing, and group drug counseling.[117] Although there are no current FDA-approved therapeutical treatments for CUD, several medications have been used for their off-label effects on patients who do not respond well to psychosocial therapies.

For instance, topiramate, an anti-seizure medication that activates GABA and decreases dopamine release, is sometimes used at low doses to augment CUD psychosocial treatment.[117,118] Some studies find it more effective than a placebo in reducing weekly cocaine use and suppressing the reinforcing effects of cocaine.[119] However, conflicting clinical trial results indicate that further research is needed to confirm its effectiveness. Another option for patients unresponsive to topiramate is long-acting amphetamines. Although clinical trials did find them effective in reducing cocaine use, most trials reported poor retention rates. In addition, as a DAT inhibitor similar to cocaine, amphetamine's risk of misuse cannot be overlooked.[117]

Another medication, disulfiram, traditionally used to treat alcohol dependence, has shown some efficacy in increasing abstinence rates compared to a placebo. However, it does not significantly affect the frequency or amount of cocaine use compared to placebo, although it does reduce the frequency of cocaine use when compared to naltrexone.[23,120]

Ketamine, however, has several potential advantages for managing CUD. Like all other medications mentioned, ketamine has been shown to reduce cocaine cravings and promote abstinence in multiple clinical trials.[121] In the meantime, its rapid antidepressant effects[120] may help CUD patients cope with withdrawal-related depressive symptoms, aiding in remission. In addition, ketamine targets NMDARs to modulate the DAergic pathway, offering an alternative for individuals unresponsive to other treatments. Therefore, compared to other treatments often associated with barriers such as stigma, addiction risk, and limited accessibility,[119] ketamine's unique properties and rapid action make it a promising alternative for reducing the harm associated with CUD.

9. Conclusion

Subanesthetic ketamine shows promise as a pharmacological intervention for CUD. Growing evidence highlights its ability to reduce cocaine consumption in dependent individuals. Pre-clinical research has demonstrated that a single subanesthetic dose of ketamine can reduce cue-induced craving, suppress drug-seeking behaviors, and increase executive control over reward-based decision-making.[22,19] Furthermore, human subjects exhibited decreased cocaine self-administration, craving, and enhanced motivation to quit cocaine after a single ketamine infusion.[31,24]

Although ketamine reduces cocaine misuse, there is mixed evidence that subanesthetic ketamine leads to long-term abstinence. However, recent findings suggest that a reduction in cocaine consumption is as clinically significant as achieving abstinence.[113,114] Thus, even if sobriety remains the ultimate objective, reduced cocaine use should be considered a valuable measure of improvement in CUD treatment.

Despite ketamine's potential for abuse,[26] its context-dependent nature allows for the safe administration of subanesthetic ketamine to individuals with CUD under the supervision of health-care professionals, similar to the use of Spravato as depression therapy.[19,26,122] Before integrating subanesthetic ketamine as a harm-reduction strategy for CUD into clinical practice, additional research must delineate ketamine's mechanism of action against cocaine. Current research suggests that ketamine may alleviate CUD by modulating the dopaminergic system by altering dopamine levels directly, increasing BDNF

levels, or blocking LHB-bursting neurons. In addition, pre-clinical research shows sex-dependent differences in ketamine response, although the mechanism responsible for this phenomenon is still unclear.

Despite the significant advancement in ketamine research, most studies relied on anti-depressant animal models. To explore ketamine's full potential in treating CUD, more clinical and pre-clinical studies should focus specifically on ketamine's mechanisms and effects using a CUD model. In addition, future investigations should also establish optimal dose and frequency schedules to maximize patient safety and foster durable therapeutic outcomes.[119]

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Conflict of interest

The authors declare they have no competing interests.

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REVIEW ARTICLE

Opioid use among Nigerian students: Exploring knowledge gaps and preventive strategies

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Abstract

The increasing rate of opioid consumption among Nigerian students is alarming. Despite the therapeutic benefits of opioids, their excessive use could be detrimental. Exploring knowledge gaps and implementing preventive strategies are necessary to curb opioid misuse among Nigerian students. This review aimed to identify the prevalence estimates of non-prescribed opioid use among Nigerian students and explore the cultural and societal factors favoring opioid and other drug use in this population. It further aimed to provide and discuss strategies that could offer effective and long-lasting solutions for Nigerian policymakers to curb opioid use among Nigerian students. A systematic search strategy identified relevant studies from databases such as PubMed and Google Scholar using keywords such as opioid misuse, students, Nigeria, prevention strategies, and opioid use disorder. Articles on the prevalence rates, associated factors, and preventive measures were extracted. The findings revealed a high prevalence of opioid use among Nigerian students, particularly in the southwestern (7.87%) and northeastern (6.57%) parts of Nigeria. Approximately 4.6 million Nigerians, constituting 4.7% of the population, have used opioids for non-medical purposes. Tramadol and codeine are the most commonly misused opioids. Cultural practices and belief systems often impact the knowledge, attitudes, and perceptions of opioid use among students. Efforts to prevent and control opioid misuse in Nigeria should focus on effective coordination among students. Health-promotion measures and specific protection should be promoted alongside early diagnosis, prompt treatment, and rehabilitation of affected individuals.

Keywords: Opioid misuse; Students; Nigeria; Prevention strategies; Opioid use disorder

1. Introduction

Opioids are pain-relieving drugs with severe side effects such as addiction potential, poisoning, and overdose. In 2021, the Centers for Disease Prevention and Control (CDC) of the United States reported that one-third of drug overdose deaths involved opioids.[1] This translates to approximately 220 deaths caused by opioid overuse per day.[1] The increased craving and non-medical utilization of opioids among students may be attributed to the rise in opioid prescriptions such as analgesics.[2] Students who misuse opioid prescriptions are more prone to alcohol and marijuana consumption.[3] In addition, factors such as distress, depression, and suicidal tendencies predispose students to opioid misuse.[4] How students obtain opioids is also a point of interest, with the main sources being their homes and friends.[5] The terms used for opioid-related issues include opioid use disorder (OUD), opioid misuse or opioid dependence, or opioid addiction.[1] Due to the risks associated with its misuse, the CDC focuses on prescription monitoring, state laws, education, quality improvement, and awareness.[1] Adequate reporting of data regarding its prevalence will be beneficial for its prevention. Despite studies on opioid use in Nigeria, only a few focused on Nigerian students, particularly identifying prevalence estimates of non-prescribed opioid use among Nigerian students and exploring cultural and societal factors favoring opioid and other drug use in this population. In addition, studies on prescription drug use and illegal opioid use are limited. This review aimed to identify the prevalence estimates of non-prescribed opioid use among Nigerian students and explore the cultural and societal factors favoring opioid and other drug use in this population. It further seeks to provide and discuss strategies that could offer effective and long-lasting solutions for Nigerian policymakers to curb opioid use among Nigerian students.

2. Methodology

A systematic search was performed across electronic databases such as PubMed, Scopus, Google Scholar, and ResearchGate using the following Boolean operators: (Opioid Misuse) AND (Students) AND (Nigeria) AND (Prevention Strategies) AND (Opioid Use Disorder). Studies on opioid misuse among Nigerian students were identified. Articles were selected based on their relevance, focusing on prevalence rates, associated factors, and preventive measures. The inclusion criteria were as follows: English language articles published between 2008 and 2024 that focused on opioid misuse among Nigerian students, original research articles, reviews, case studies, cross-sectional studies, randomized control, non-randomized control trials, and case reports, and studies on Nigerian students involved in substance abuse. The exclusion criteria were as follows: non-English language articles, articles with no full text available, duplicate publications or studies with overlapping data published before 2008 or after 2024, articles that did not focus on opioid misuse or substance abuse, editorials, letters, case reports, non-human studies or studies not focused on Nigerian students, and articles on diseases not relevant to Nigeria.

3. Opioid misuse among Nigerian students

In Nigeria, a student usually aged 18 – 29 years old is described as someone who is enrolled in formal education at various levels, ranging from primary to tertiary and even vocational institutions, to gain information and skills in various subjects of study. Nigerian students, such as students elsewhere, experience academic hurdles, social pressures, and health problems. Akande-Sholabi *et al*. (2019) reported a significant health issue among Nigerian students, namely opioid use, particularly with codeine-containing drugs. In Nigeria, cough syrups include codeine, a commonly misused narcotic that students ingest.[6] Despite its prohibition in several countries, including Nigeria, codeine misuse has become a widespread trend among Nigerian youngsters and is frequently a gateway to the misuse of other narcotics.[7]

The opioid crisis is a worldwide problem, and Nigeria is no exception. Opioid misuse among Nigerian students has been connected to several causes, including easy access to narcotics, peer influence, and the desire to cope with academic stress and social issues.[8] According to Akande-Sholabi *et al*. (2019), codeine-containing cough syrups are the most frequently taken opioid medicines among Nigerian students, underlining the issue of drug use in educational settings. The misuse of opioids endangers students' health and well-being, resulting in addiction, negative impacts, and potential long-term implications.[9]

The availability and accessibility of opioids in Nigeria contribute significantly to student drug misuse. Despite the country's opioid-prescribing limitations, issues in properly implementing these rules remain, resulting in the ongoing incidence of opioid misuse.[10] Furthermore, the social acceptance and normalization of opioid use in specific situations contribute to the problem, producing a culture where substance misuse is not.[8] The lack of strict enforcement of opioid-dispensing restrictions at community pharmacies also contributes to opioid misuse in patient populations, such as individuals with sickle cell illness.[11]

Furthermore, students' attitudes and beliefs about opioids have a noteworthy influence on their overuse. Pharmacy schools should add opioid misuse management

into their curricula to teach future healthcare professionals about the hazards associated with these medicines.[12] Furthermore, students' prescription behavior has shifted, with fewer students willing to be prescribed opioids in clinical practice, indicating a rising knowledge of the hazards associated with these drugs.[13] This shift in attitude toward opioid prescription mirrors a larger trend of healthcare professionals and students adopting more cautious and educated practices.

When tackling the opioid issue among Nigerian students, sociocultural elements that affect pain treatment practices in the nation must be evaluated.[14] These issues, such as human resource restrictions, infrastructural problems, and budgetary constraints, influence effective pain management and lead to opioid misuse as a coping mechanism.[14] Furthermore, the modest presentation of OUD in some patient populations, such as those with sickle cell disease, emphasizes the significance of early diagnosis and referral to treatment facilities for appropriate management.[15]

4. Types of opioids consumed

In Nigeria, the opioid landscape is characterized by a wide range of drugs, with tramadol and codeine as the most often abused opioids. Tramadol, a synthetic opioid analgesic similar to meperidine, has become popular because it is more easily accessible and thought to be safer than conventional opioids. It is commonly used to treat moderate-to-severe pain; however, it is also becoming increasingly popular for its psychedelic properties. Studies have revealed that tramadol abuse is prevalent among young people, raising serious public health issues.[16,17]

Non-prescription opioids, also known as illicit opioids, are those that are not legally prescribed by a registered medical practitioner. They are often obtained and used illegally, usually injected, snorted, or smoked. Heroin, opium, and illicitly manufactured fentanyl are some of the most common non-prescription opioids. Some of these are illicitly manufactured or present as counterfeit pills, illicit opioids pressed into pills that mimic prescription opioids.[3,4,18] Prescription opioids are those prescribed by a registered medical practitioner. These are legally produced but are abused considerably by students with OUD. Oxycodone, hydrocodone, morphine, codeine, fentanyl, methadone, hydromorphone, oxymorphone, buprenorphine, tramadol, and tapentadol are the commonly prescribed opioids for pain but carry a risk of misuse and addiction. These drugs are also used in combination with other medications such as acetaminophen. Methadone is also used as a medication for the assisted treatment of opioid addiction.[19,20]

Codeine is another extensively abused opioid. It is commonly present in cough syrups and is easily accessible over the counter in many Nigerian pharmacies. The availability of codeine-containing products has led to its misuse, particularly among students who may use it for recreation or self-medication.[6] The "codeine diet," in which people consume significant amounts of codeine syrup combined with soft beverages, was a trend among Nigerian adolescents, illustrating the cultural norms around opioid use.[21]

Oxycodone and hydrocodone are less often reported in Nigeria due to their limited availability and stricter regulatory requirements. However, opiate abuse is not completely absent because some students may seek them out illegally for recreation or pain management.[22] The rise in polydrug use, where opioids are combined with alcohol or stimulants, complicates the opioid consumption picture among Nigerian students.[21]

5. Methods of opioid consumption

The methods of opioid use among Nigerian students vary greatly based on cultural conventions, availability, and personal preferences. Oral consumption is the most common delivery method, particularly for tramadol and codeine. Students may mix them with liquids, such as soft drinks or alcohol, to improve their effects. This strategy increases not only the risk of overdosing but also the likelihood of developing substance use disorders.[21,22]

Injecting opioids has also been documented; however, it is less frequent among the general student population. This approach is often associated with more severe substance use problems and is frequently observed among marginalized populations or individuals with a history of intravenous drug use.[23] The stigma associated with the use of injectable drugs may discourage many students from using this approach, resulting in a preference for oral use.

In addition to traditional methods of use, the trend of combining opioids with other drugs to obtain the desired effects has been increasing. For example, tramadol combined with alcohol or other stimulants is a common practice among students seeking to improve their experiences at social gatherings or parties.[21] This polydrug use has serious health hazards because the combination of multiple drugs might result in unforeseen and deadly effects.

The motives for opioid use among Nigerian students vary. Many students have reported taking opioids to deal with academic stress, emotional discomfort, or social pressure. The cultural environment in which these medications are taken significantly affects the views about opioid use. For example, the normalization of some

consuming behaviors, such as the "codeine diet," reflects broader cultural views that may minimize the dangers of opioid misuse.[21,22]

6. Side effects and adverse effects of opioid use

Although opioids are effective in managing pain, they are associated with various side effects and adverse effects that can significantly affect the quality of life of individuals, particularly among vulnerable populations such as students. Understanding these effects is crucial for developing preventive strategies against opioid misuse, particularly among Nigerian students, who may have limited awareness of the potential dangers associated with opioid use. This section delves into the side effects and adverse effects of opioid use, highlighting their implications for health and well-being.

6.1. Common side effects of opioids

Opioids have different side effects that can be classified into common and severe categories. The most prevalent side effects are nausea, vomiting, constipation, sedation, and respiratory depression. Nausea and vomiting are particularly common and affect a significant proportion of patients receiving opioid therapy. Studies have indicated that these emetic effects can lead to the discontinuation of opioid use, undermining pain management strategies.[24,25]

Constipation is another prevalent side effect, often referred to as opioid-induced constipation (OIC). OIC affects 15 – 70% of patients on opioid therapy depending on the specific opioid and dosage.[26] This condition can lead to significant discomfort and may require additional medical interventions, further complicating pain management.[27] The mechanisms behind OIC involve the inhibition of excitatory neural pathways in the gastrointestinal tract, resulting in decreased colonic propulsion.[26]

Sedation is frequently observed, particularly during the initial stages of opioid therapy or with increasing dose. This side effect can impair cognitive function and daily activities, posing risks for students who may already be dealing with academic pressures.[28,29] Furthermore, respiratory depression is a severe side effect that can lead to life-threatening situations, particularly in cases of overdose or when opioids are combined with other central nervous system depressants.[28,30]

6.2. Long-term adverse effects

The long-term use of opioids can lead to more serious adverse effects, including the development of tolerance, physical dependence, and addiction. Tolerance occurs when the body becomes accustomed to the presence of the drug, necessitating higher doses to achieve the same analgesic effect.[25,31] This phenomenon can lead to a cycle of increasing dosage and increased risk of adverse effects, including overdose.

Physical dependence is characterized by withdrawal symptoms when the drug dose is reduced or discontinued. Symptoms can vary from mild discomfort to severe physiological reactions, complicating opioid therapy cessation.[32] The psychological aspect of addiction, which includes compulsive drug-seeking behavior despite harmful consequences, poses significant challenges for individuals, particularly students who may be under stress.[31]

Moreover, chronic opioid use has been linked to cognitive decline and mood disorders, further intensifying the challenges faced by students. Research indicates that long-term opioid therapy can impair cognitive functions, leading to difficulties in concentration and memory, which are critical for academic success.[29,33]

6.3. Impact on the quality of life

The side and adverse effects of opioids can profoundly affect an individual's quality of life. For students, the combination of pain management and side effects of opioids can decline academic performance and social interactions. The stigma surrounding opioid use, particularly within cultural contexts like Nigeria, may further isolate individuals who struggle with opioid-related issues.[34,35]

In addition, the financial burden associated with side effect management – such as the need for laxatives to counteract constipation or additional medical consultations – can strain the resources of students and their families.[27,36] This economic effect can deter students from seeking help or adhering to prescribed therapies, perpetuating a cycle of misuse and adverse outcomes.

7. Prevalence of opioid use among Nigerian students

Several studies in Nigeria have provided substantial data on opioid use among Nigerian students. For example, a cross-sectional study from the University of Fort Hare and Ondo State University in Nigeria showed that nearly one-fourth of students (24.5%) had engaged in recreational drug use and approximately 17.5% were still involved.[7] Another cross-sectional study from the University of Ibadan, Nigeria, in 2018 reported that more than one-third of the students (34.2%) had used opioids.[6] A study from Ilorin, Kwara State, Nigeria, reported that approximately 40% of students aged 10 – 19 years were involved in drug use, where approximately 4% of the selected students had already used heroin.[37] Heroin is an illicit opioid, mainly

used as a non-prescription opioid. A study based on government schools in Lagos, Nigeria, with students aged 10 – 15 years, reported that opioids had a 9% lifetime drug prevalence rate.[38] Opioid use was found to be associated with sex, education, school management, and economic status.[38] Most Nigerian students reported being introduced to opioids by friends, with only a few being introduced by family or relatives. Students also reported consuming opioids at parties because of peer pressure[6] and curiosity.[39] Most students believed that counseling could help address this situation[39] (Figure 1).

8. Cultural influences on opioid use in Nigeria

According to the United Nations Office on Drugs and Crime, approximately 4.6 million Nigerians, constituting 4.7% of the population, have used opioids for non-medical purposes.[40] Tramadol and codeine are the most commonly misused opioids.[40] Tramadol has become a widespread concern because of its availability and affordability. In Nigeria, attitudes and values surrounding drug use, particularly opioid use, are significantly influenced by the complex interactions among cultural norms, societal expectations, and modern influences.[41] Cultural practices and belief systems, which socially integrate alcohol and drug use, often shape these attitudes.[42] Although drug use is generally considered taboo in Nigeria and is discouraged because of its association with health risks, addiction, and societal problems, its prevalence and effect cannot be overlooked. A concerted effort has been made to inform children in Nigerian schools about the risks associated with substance misuse.[43] Through awareness campaigns and counseling sessions, school administrators and teachers strongly emphasize prevention, highlighting the detrimental effects on the academic performance and general well-being of students.[44]

The digital media in Nigeria typically portrays drug use negatively,[45] exposing its destructive consequences through news reports, public service announcements, and entertainment content. Parents and teachers take on vital roles in discouraging drug use among Nigerian youth, emphasizing the importance of making healthy choices and seeking help if needed.[46] Despite these efforts, societal pressures and peer influences may glamorize drug use, particularly among youths.[47] Research consistently shows that social and peer influences play crucial roles in the initiation and continuation of drug use among adolescents.[48] Some individuals may feel compelled to experiment with drugs to fit in with certain social circles. However, those who choose not to use drugs often rely on strong family values and educational messages that promote resilience and self-worth. The influence of modernity, including urbanization and exposure to global trends, has affected attitudes toward drug use in Nigeria. Klein and Fiki highlighted that the spread of drug use, including opioids, among Nigerian youth is due to increased exposure to

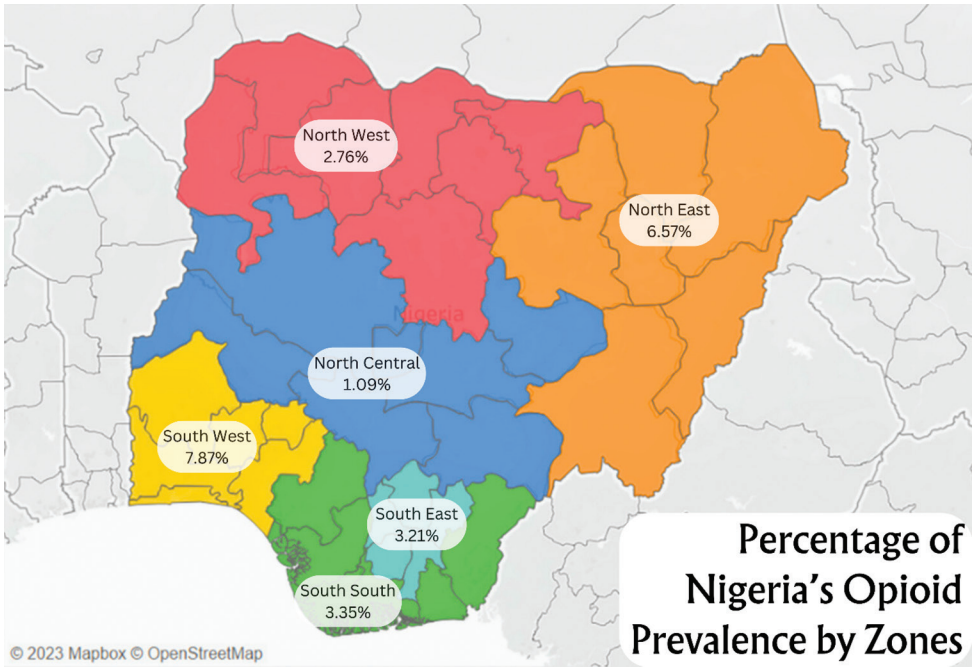

Figure 1. Percentage of Nigeria's Opioid Users in each of the six geographical zones. Map data made available from ©OpenStreetMap under the Open Database License.

Western influence.[49,50] This is further exacerbated by the neglect of rural communities and the activities of untrained individuals in the rural health sector.[49] Although the traditional values of community, family, and spirituality continue to shape societal norms, increasing access to technology and exposure to Western media may contribute to changing perceptions of substance use among members of the population. In addition, the rural-urban differences in attitudes toward modern technological gadgets, such as the global system of mobile communication, may play a role in shaping attitudes toward opioid use among Nigerian students.[51] The persistent stigma surrounding addiction is one of the challenges in addressing drug use in Nigeria. Ebigbo and Molobe highlighted the unique forms of cultural taboo and stigma faced by female African drug misusers, which can restrict their access to treatment.[52,53] Individuals and families affected by drug addiction may hesitate to seek help due to fear of judgment or discrimination. However, growing initiatives and advocacy efforts are aimed at reducing stigma and promoting open discussions about addiction and mental health in the country.

9. Strategies to curb opioid use among Nigerian students

9.1. Actions by the government and international bodies

Curbing opioid use in Nigeria requires a multifaceted approach involving government intervention, community engagement, education, accessible treatment options, and a concerted effort across all social classes. At the government level, laws and regulations related to opioid use in Nigeria have been enforced. The National Agency for Food and Drug Administration and Control regulates the distribution and use of opioids and other controlled substances in Nigeria.[54] Nigeria's drug laws and regulations are overseen by the National Drug Law Enforcement Agency (NDLEA). The NDLEA is tasked with reducing the unlawful manufacture, import, export, sale, and trafficking of psychoactive substances.[55] However, enforcing these regulations and combating illicit drug trafficking remains a challenge.

9.2. Actions by schools and institutions

Some non-profit organizations in Nigeria have been proactive in addressing drug misuse, particularly among adolescents, by implementing prevention programs in schools and communities to raise awareness about opioid misuse. These programs often target schools and communities to educate individuals about the risks of OUD and provide information on where to seek help. School-based substance use interventions have proven effective when implemented using various strategies.[56] Prevention and awareness programs have been conducted in Nigerian schools to educate students about the dangers of opioid use. These programs typically involve presentations, workshops, and counseling sessions led by health professionals or community organizations. In schools, counseling services may be available to students struggling with substance misuse. However, the availability and quality of these services can vary depending on school resources. Community-level interventions are crucial for effectively addressing OUD and reaching a broader audience in understanding the effect of OUD. Engaging Nigerian community leaders, religious institutions, and local organizations in awareness campaigns and support networks can help reduce stigma and promote the understanding of addiction as a treatable health condition.

9.3. Actions at the community level

In Nigeria, treatment options for OUD include counseling, detoxification, and rehabilitation programs.[57] Behavioral therapies and medication-assisted treatment may be available in specialized addiction treatment centers.[58] However, access to these services can be limited, particularly in the rural areas of Nigeria. Rehabilitation residences in Nigeria play a crucial role in supporting individuals recovering from OUD. Recovery residences may increase an individual's recovery capital, facilitate social support for abstinence, and foster a sense of community among residents.[59] Although the behavior of these residents varies, ideally, they should provide comprehensive care, including medical supervision, counseling, and vocational training to help individuals reintegrate into society. Challenges such as funding constraints and lack of trained personnel can affect the quality of care provided.

Access to help for OUD varies across different settings in Nigeria. Accessing treatment for OUD may be more challenging in rural or underserved areas because of limited healthcare infrastructure.[60] To ensure the effectiveness of efforts to reduce opioid use, ongoing monitoring and evaluation of prevention programs and treatment services are essential.[61,62] This includes collecting data on substance misuse trends, assessing the effect of interventions, and identifying areas for improvement or expansion of services. Strategies to curb opioid use in Nigeria require a coordinated effort involving government policies, community engagement, accessible treatment options, awareness programs, and ongoing education.

10. Global comparisons of opioid use in other countries

The global landscape of opioid use is diverse, with significant differences in consumption patterns, regulatory

frameworks, and public health effects among countries. Although opioid misuse is frequently linked to the United States due to its vast epidemic, this problem is a major concern in several other nations, albeit in different contexts and influences. This section examines opioid consumption patterns in several nations, highlighting major distinctions and parallels to the situation in Nigeria, and the larger implications for global public health.

The opioid problem in the United States has reached frightening proportions, with a sizable section of the population suffering from OUD. The crisis was partly caused by the aggressive marketing of prescription opioids in the late 1990s and early 2000s, which minimized the risks of addiction. Consequently, opioid prescriptions skyrocketed, causing a concurrent increase in addiction and overdose mortality. By 2019, opioids accounted for >70% of drug overdose deaths in the country.[63] The United States' experience demonstrates the hazards of overprescription and the need for strict regulatory control to avoid abuse.

In contrast, several European countries have practiced more careful opioid prescribing practices, resulting in lower rates of drug abuse and addiction. For example, a study of opioid consumption trends in Europe from 1990 to 2016 discovered that despite the increase in opioid use for pain management, misuse, and addiction rates remained much lower than that in the United States.[64] This might be ascribed to stricter regulatory frameworks and a more conservative attitude to pain treatment among European healthcare practitioners. Sweden and Norway have strong public health policies that promote non-opioid pain care options, which contribute to their effectiveness in preventing opioid use.[65]

However, the situation in Eastern Europe is more complicated, with Romania and Bulgaria having higher opioid misuse rates. This issue is frequently connected to socioeconomic situations and underfunded healthcare systems, leading to restricted access to legally prescribed opioids and a consequent reliance on illicit sources of pain management.[66] The variations in opioid availability and usage across Eastern and Western Europe highlight the importance of tailoring public health interventions to each region's specific difficulties.

Opioid use has historically been minimal in Asia, notably in Japan, due to cultural views toward pain treatment and stringent regulatory constraints. Recent researchers have found that despite the rising acknowledgment of chronic pain as a serious health problem, Japan has largely avoided the tremendous growth in opioid use seen in other countries.[67] However, there is still cause for concern because of the prescription opioid misuse and abuse rates of 45.5% and 24.5%, respectively, highlighting the need for increased awareness and preventive actions among healthcare practitioners.[18]

The situation in developing countries, such as Nigeria, is very different. Its opioid misuse is frequently connected to a lack of knowledge and education about the hazards associated with these drugs. Medical and pharmacy students in Nigeria consume codeine at high rates because of social pressure and a lack of education about the consequences of opiate usage.[68] This demonstrates a serious information gap and the need for comprehensive preventive interventions to address the growing trend of opioid misuse among Nigerian students.

Cultural variables also have a substantial effect on the views about opioid use in Nigeria. Because opioids are widely available and used in conventional medicine, many people believe they are harmless. This cultural acceptability can contribute to the higher drug abuse rates because people may not fully appreciate the risk of addiction and other negative consequences of opioid use.[69] Addressing these cultural elements is critical to the development of successful public health programs in Nigeria.

Globally, the World Health Organization (WHO) has underlined the significance of balanced pain treatment techniques that consider both the therapeutic advantages and hazards of opioid addiction. The WHO promotes the development of national policies that encourage safe prescription practices, public education campaigns, and greater access to addiction treatment services.[19] Such activities are critical in combating the global opioid issue, particularly in countries such as Nigeria, where a lack of knowledge and education adds to increased drug abuse rates.

11. Future directions and research needs

As the opioid crisis continues to spread worldwide, future paths and research requirements must be identified to effectively address the issues posed by opioid use, particularly among vulnerable groups such as students and youth. This section discusses critical topics for future research and intervention techniques that will help us gain a better knowledge of opioid use and prevention.

First, longitudinal studies that monitor opioid use patterns over time are urgently needed, particularly among teenagers and young adults. Such studies can provide important insights into the elements that contribute to the onset and course of opioid misuse. For example, a study conducted in the United States found that early exposure to prescription opioids during adolescence increases the risk of developing OUD later in life.[70] A similar study in

Nigeria could explore the trajectories of opioid misuse among students, identify important intervention points, and inform targeted preventive efforts.

In addition to quantitative research, qualitative studies are warranted to gain a better understanding of the cultural and socioeconomic forces that determine the views regarding opioid use among Nigerian students. Researchers can utilize approaches such as focus groups and interviews to investigate students' perspectives, beliefs, and experiences with opioid use and misuse. This qualitative technique can reveal the underlying motives for opioid misuse, including peer pressure, academic stress, and desire for pain relief, which may differ dramatically from those reported in Western settings.[68]

Furthermore, preventive treatments that are adapted to Nigeria's specific cultural and socioeconomic circumstances are needed. Programs that include community participation, education, and awareness campaigns can help reduce the stigma associated with opioid addiction and promote healthy coping techniques among adolescents. For example, peer-led education and support groups may promote a feeling of community while encouraging frank talks regarding substance use and mental health. Such interventions should be culturally sensitive and tailored to the unique values and conventions of the Nigerian population.

Research should also examine the effectiveness of Nigeria's current opioid-prescribing guidelines and laws. Understanding how these regulations affect opioid availability and usage can inform future policy decisions and reveal gaps in the existing regulatory system. Comparative research on the success of opioid policy in other countries could offer valuable insights to Nigeria as it develops a comprehensive strategy for opioid management.[19] For example, Canada's opioid-prescribing guidelines, which emphasize non-opioid alternatives and holistic pain management practices, could serve as a model for reducing addiction risks while ensuring effective pain treatment tailored to the Nigerian context.

The application of technology in opioid misuse prevention represents an intriguing area for future study. Mobile health applications and online platforms may be used to disseminate instructional content, provide resources for students, and improve access to mental healthcare. Research on the feasibility and effectiveness of such digital interventions in the Nigerian setting can help in the development of creative strategies for combating opioid usage among students.[66] These technological tools can also be used to track opioid use trends and identify at-risk individuals, allowing for early intervention and assistance.

Finally, coordination among academics, healthcare providers, politicians, and educational institutions is essential

Table 1. Summary of the actions taken to control opioid use among Nigerian students

Heading	Actions taken to control opioid use
Government efforts	The NAFDAC oversees opioid distribution, whereas the NDLEA enforces laws and fights illegal trafficking. They provide funding for prevention initiatives, treatment centers, and law enforcement while developing and updating laws to control opioid production, distribution, and prescription. [25]
Community initiatives	Mobilizing community leaders, religious groups, and local organizations to educate the public and combat stigma; setting up community support groups to assist those affected by opioid addiction; and involving community members in planning and executing culturally relevant interventions. [8]
School-based strategies	Organizing workshops, presentations, and counseling sessions by health experts to warn students about opioid dangers; offering counseling and mental health services in schools for students dealing with substance abuse; and encouraging student-led support groups to promote healthy choices and mutual support. [15]
Parental roles	Teaching parents to recognize signs of opioid misuse and understand the associated risks, promoting honest conversations between parents and children about drug use, and encouraging parents to supervise activities and provide emotional support to help children resist peer pressure. [17]
Educational institution's role	Including substance abuse education in school curricula for comprehensive student knowledge, equipping teachers and staff with the skills to identify and address opioid use among students, implementing policies, and establishing and enforcing school policies against drug use while supporting affected students. [15]
International organization's support	Offering technical expertise and resources for effective prevention and treatment programs, providing funding for local efforts to reduce opioid use and enhance healthcare infrastructure, partnering with Nigerian institutions for research, and sharing best practices in combating opioid misuse. [25]
Healthcare provider's contribution	Ensuring availability of counseling, detoxification and rehabilitation services, particularly in rural areas; providing behavioral therapies and medication-assisted treatments in specialized centers; and training healthcare workers to manage opioid addiction cases effectively and compassionately. [14]

Abbreviation: NDLEA: National drug law enforcement agency.

to develop a comprehensive plan for combating opioid use. Multidisciplinary methods that include stakeholders from multiple sectors can improve the efficacy of preventive and intervention activities. For example, collaborations between universities and healthcare institutions can help design comprehensive educational programs that provide students with the required knowledge and skills to manage the intricacies of opioid use and misuse.[20] Furthermore, multinational cooperation can facilitate information exchange and sharing of best practices, boosting the global response to the opioid pandemic (Table 1).

12. Conclusion

This review highlights the multifaceted nature of opioid misuse among Nigerian students, involving social, cultural, economic, and personal factors. Addressing this issue requires intersectoral coordination among the government, healthcare providers, communities, and educational institutions. Effective coordination is important for implementing opioid prevention and protection measures in Nigeria should be channeled through. Strategies should include health promotion, targeted protection, early diagnosis, prompt treatment, and rehabilitation of affected individuals. Despite some progress in preventing opioid misuse in Nigeria, it efforts are often hindered by insufficient law enforcement and healthcare facilities. Educational programs, along with rehabilitation efforts such as counseling or peer support groups, should be equally prioritized. Moreover, ongoing research, including cohort studies, case reports, and cross-sectional studies, is necessary to generate accurate data on knowledge, awareness, practice, and prevalence of the issue. This narrative review will contribute to the quality assessment of opioid misuse prevention, particularly in Nigeria, and help combat the issue effectively.

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Author contributions

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ACCSCIENCE PUBLISHING

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REVIEW ARTICLE

Leveraging bibliometric analysis competencies for improved postgraduate pharmaceutical education in China

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Abstract

Postgraduate pharmaceutical students are at the forefront of drug development and application in China. In recent years, global pandemics, demand for the research and development of new drugs, and college and university requirements of outcome-based education (OBE) indicate that major reforms in postgraduate pharmaceutical education are essential. A currently neglected aspect of OBE in Chinese pharmaceutical education is bibliometric analysis. To establish a framework for bibliometric analysis training, the researchers searched the top 50 research works in major academic databases using the keywords "Pharmacy education" and "Framework." Ten papers were selected that were closest to the desired paradigm. The procedures, theoretics, and goals of the frameworks in these papers were combined with the researchers' teaching and research experience to construct a bibliometric analysis competency (BAC) framework. The BAC framework, which was created to fill current gaps in postgraduate pharmaceutical education, constitutes three levels: (A) knowledge; (B) familiarization; and (C) mastering. The three levels require graduate students to (A) have a preliminary understanding of the steps of bibliometrics, (B) comprehend the literature on bibliometrics, and (C) independently apply the steps to perform bibliometric analysis, respectively. This paper also describes the theoretical setup of the BAC framework and a potential assessment system. The BAC framework provides a useful reference for the reform of Chinese pharmaceutical graduate education, and its future implementation is urgently anticipated.

Keywords: Bibliometric analysis competencies; Framework; Pharmaceutical education; Postgraduate education; China

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1. Introduction

Since ancient times, China has attached great importance to the research, development, and application of medicines, forming a unique traditional Chinese medicine (TCM) system that involves botanic (such as the roots and seeds of *Arctium Lappa*[1]), animal (such as the musk of *Moschus berezovskii Flerov*, *Moschus sifanicus Przewalski*, or

Moschus moschiferus Linnaeus[2]), fungal (such as *Cordyceps sinensis*[3]), and mineral (such as cinnabar[4]) ingredients. Before the introduction of Western medicine, TCM was the main means of disease treatment in China. Since the introduction of the Western medicine system into China, China has also begun to advance the development and application of modern drugs, including small molecule chemical drugs,[5] biological macromolecular drugs (proteins, polysaccharide, nucleic acid, etc.),[6] and vaccines.[7] In recent years, the concept of biotherapy has also received increased attention in China. For instance, the most famous biotherapy approaches, chimeric antigen receptor (CAR)-T-cell therapy and CAR-NK cell therapy, are promising new methods of cancer treatment.[8]

The development and application of both TCM and modern medicine require pharmaceutical knowledge, which substantially depends on the cultivation of pharmaceutical talent. This cultivation is realized mainly through pharmaceutical education. Throughout history, Chinese culture has attached importance to pharmaceutical education, training numerous talented Chinese pharmacists. Sun Simiao, who presided over the world's first national pharmacopeia, *Tang Xin Materia Medica*, is well known in China to this day.[9] Furthermore, Li Shizhen's *Compendium of Materia Medica* is known as the "great book of oriental medicine."[10]

In ancient times, the cultivation of pharmaceutical talents mainly depended on the master–apprentice system. In modern China, however, the training of pharmaceutical talent has gradually shifted to the formal modern college pharmaceutical education system. In this system, postgraduate education occupies a very important position. This is because its key focus is to train masters and doctors with independent scientific research abilities and provide fresh forces for the field of drug design and development.[11] However, other components of the modern college pharmaceutical education system – that is, vocational and undergraduate education – do not focus on this aim (Figure 1). Postgraduate education is indispensable and irreplaceable for future pharmacists.

Nowadays, the main curricula for pharmacy postgraduates in China constitute some theoretical courses (such as pharmacology or pharmaceutics), experimental curricula (such as drug analysis and medicinal chemistry experimentation), and practical courses (such as internships); however, no course focuses on bibliometrics training specifically. As a result, students' abilities to analyze the existing literature and understand the status of current research, hotspots, and development trends in the pharmaceutical field are not being efficiently cultivated.

2. Background

2.1. Why propose a new framework?

With the development of the economics and technologies, the demand for health in human society is increasing, which puts forward higher requirements for the field of drug research and development. In this context, improving the training quality of postgraduate pharmaceutical students is necessary to cultivate stronger independent scientific research capabilities. This growing need is manifested at the following three levels (Figure 2):

(1) Societal level. In the context of the continuous recent increase in the incidence of tumors, cardiovascular diseases, and neurodegenerative diseases – as well as epidemics and pandemics such as COVID-19,[12] influenza A,[13] and monkeypox[14] – the demand for safe, effective, and quality-controlled drugs is increasing. With the discovery of new therapeutic targets for known diseases and the emergence of new infectious diseases, the pace of drug research and development is accelerating, and the demand for new drug development capabilities has increased significantly.

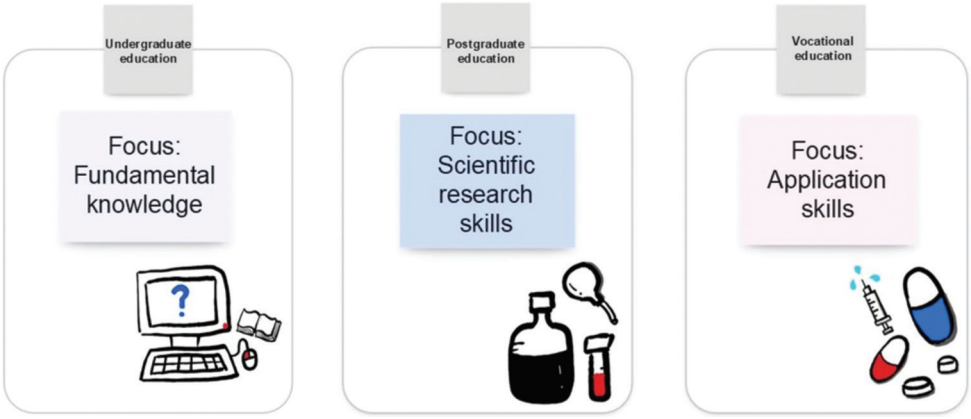

Figure 1. Key focus of each component of the modern college pharmaceutical education system

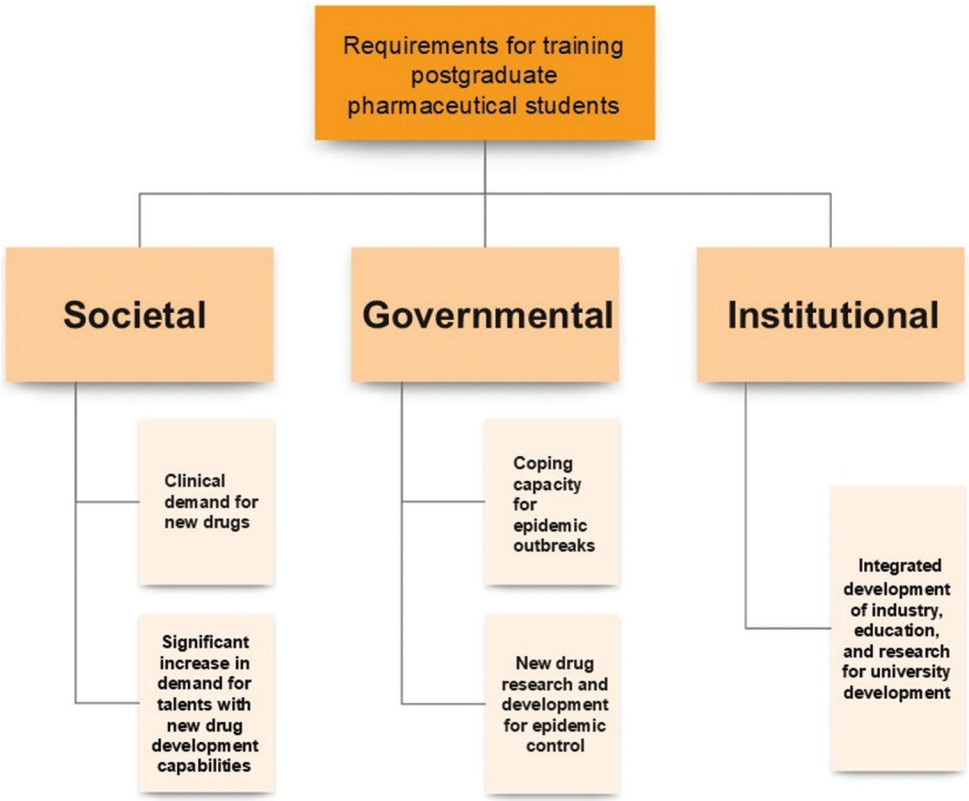

Figure 2. Requirements for training postgraduate pharmaceutical students at various levels

(2) Governmental level. Under the current situation, the ability of countries and regions to respond quickly and effectively to public health emergencies has become an important component of national system, and it is also a concrete manifestation of soft power. Taking the fight against the COVID-19 epidemic as an example, the research and development process of preventive vaccines,[15] diagnostic reagents,[16] and therapeutic drugs[17] has largely determined the effectiveness of epidemic and pandemic control. This also requires the support of new drug research and development.

(3) Institutional level. The integrated development of industry, education, and research is one of the mainstream ideas in the contemporary development of colleges and universities.[18] In the context of the growing industrial demand for drug research and development capabilities, and based on the teaching concept of outcome-based education, colleges and universities should pay more attention cultivating the independent scientific research abilities of postgraduate pharmaceutical students.

To improve the quality of postgraduate pharmaceutical training, effective education reforms are necessary to enable the cultivation of students' independent scientific research abilities and improve their competitiveness in the job market. Such reform will also hold significance for teachers as it will enhance their teaching skills. In this context, a new framework should be proposed as a theoretical guide for the corresponding pedagogical reform.

2.2. What is bibliometric analysis?

Bibliometric analysis competencies (BAC) must be part of the above-mentioned education reform and can assist in the proposal of a corresponding framework. Bibliometrics is an interdisciplinary approach in library and information management that uses both qualitative and quantitative analysis to describe and analyze the current status of scientific research in specific fields, including the number of papers, high-yield institutions, high-frequency hot words, research frontiers, and future trends. The results of bibliometric research are usually expressed in the form of graphs.[19,20] Correspondingly, bibliometric analysis refers to the following processes: (1) determination of research aim; (2) establishment of retrieval strategy; (3) literature survey; (4) software processing; (5) data interpretation; and (6) description of the knowledge domain[21] This process is illustrated in Figure 3.

Bibliometric analysis was first applied to the field of library science and information management. After years of

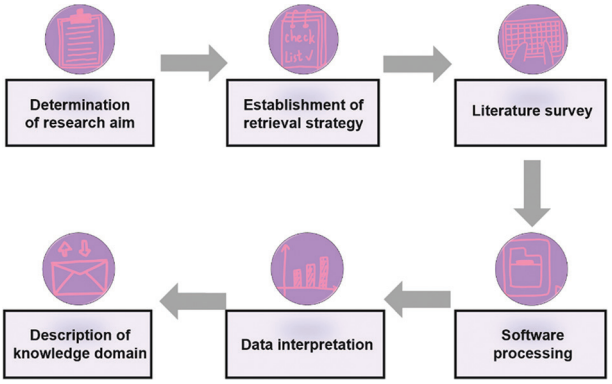

Figure 3. Bibliometric research process flowchart

development, bibliometric analysis has been widely applied to several sub-disciplines in the medical field, including physiology,[22] toxicology,[23] clinical medicine,[24] clinical chemistry,[25] public health,[26] nursing,[27] and pharmacy.[28] In the field of pharmacy, bibliometric analysis can be applied to research on various aspects of drug development, such as drug discovery,[29] medicinal chemistry,[30] natural product extraction,[31] biopharmaceutics,[32] pharmaceutical analysis,[33] pharmacology,[34] pharmaceutics,[35] pharmaceutical engineering,[36] and pharmaceutical management.[37]

Bibliometric analysis has positive implications for the field of drug discovery, and the use of bibliometric principles to evaluate, analyze, and provide historical perspective on journal evaluation has become widespread.[38] Bibliometrics has also positively influenced drug development. Bibliometric methods can be used to analyze the research status, hotspots, and development trends of drugs already on the market, which, in turn, can illuminate new directions for further research and development.[39,40] Pharmacy postgraduates, as future drug developers and researchers, should therefore master this skill to help them in their work. This article, therefore, proposes a new BAC framework as a theoretical guide to implement bibliometric training in pharmaceutical education.

3. Methods

Based on the above background, this paper proposes a framework for bibliometric analysis training. The researchers have developed the BAC framework to improve this aspect of graduate-level pharmacy education in China. The objective of this paper was to systematically analyze the existing educational resources and research findings using this framework to identify the key issues and potential areas of improvement in pharmacy postgraduate education and provide a scientific basis and guidance for future educational policies and curriculum design.

To this end, the researchers searched the literature in academic databases and search engines, including Web of Science, PubMed, Scopus, and Google Scholar on January 8, 2024, at 10 a.m. (Beijing time) using the keywords "Pharmacy education" and "Framework." Access to these databases was provided by Jinan University. The retrieved literature was sorted by relevance, and the research team downloaded the top 50 relevant papers from each search engine and read them after deduplication. The 10 papers[41-50] that were closest to the expected research paradigm of this paper (Table 1) were selected and the approaches, theoretics, and goals of these frameworks were analyzed. The team then constructed the BAC framework based on the findings as well as their own years of experience as educators and researchers.

4. Framework proposal

4.1. Tabular and schematic summary

Regarding the proposed framework, tabular and schematic summaries are provided in Table 2 and Figure 4, respectively.

4.2. Description

In this subsection, the BAC framework is explained in detail to provide some reference for the reform of pharmaceutical graduate education in China.

Based on the six technical steps of bibliometric analysis mentioned above (the determination of a research aim; the establishment of a retrieval strategy; the literature survey; software processing; data interpretation; and the description of the knowledge domain), the BAC framework is divided into three levels: A, knowledge; B, familiarization; and C, mastering (Table 2).

The internal logical relationship of the BAC framework is shown in Figure 4. Levels A–C are progressive, aligning with the principles of human cognition and higher education, and exhibit high rationality. In addition, Figure 4 shows the expected progress of the implementation of the BAC framework, which is based on a 3-year, six-semester postgraduate pharmaceutical program. In Semester 1, the pedagogy for Level A of competency is carried out as early as possible. In Semesters 2 and 3, the staff helps develop Level B competencies among postgraduate students. Based on this foundation, the remaining Semesters 4 – 6 can be used to implement the Level C training plan. Postgraduate students in other long-term study programs can make appropriate adjustments in the above schedule. A shorter study program would shorten the overall training cycle, especially Levels A and B, but should guarantee adequate training for Level C competencies. Meanwhile, a longer

Table 1. Previous studies on pharmaceutical education frameworks

No.	Journal	Title	Year of Publication	Framework Proposed	Reference
1	*Journal of Pharmaceutical Policy and Practice*	Clinical preceptor competencies for a better pharmacy education: a suggested framework for Lebanon	2020	Clinical preceptor competencies framework	Zeitoun *et al.*[41]
2	*Research in Social and Administrative Pharmacy*	Using critical realism as a framework in pharmacy education and social pharmacy research	2012	Critical realism framework	Oltmann *et al.*[42]
3	*American Journal of Pharmaceutical Education*	Entrustable professional activities as a novel framework for pharmacy education	2018	Entrustable professional activities framework	Jarrett *et al.*[43]
4	*British Pharmacological Society*	Design of a foundational sciences curriculum: applying the ICAP framework to pharmacology education in integrated medical curricula	2021	ICAP framework	Quesnelle *et al.*[44]
5	*Pharmaceutical Education*	Framework for action to implement interprofessional education and collaborative practice in pharmacy and allied health sciences programs in India	2016	Interprofessional and collaborative practice framework	Rajiahand Maharajan *et al.*[45]
6	*Pharmacy*	Development and refinement of a matrix competency framework, with associated entrustable professional activities, to support initial pharmacy education in Kuwait	2023	Matrix competency framework	Moreau *et al.*[46]
7	*Pharmacy*	Exploring the implications of a needs-based pharmacy education framework modeled on population health: perspective from a developing country	2019	Needs-based pharmacy education framework	Bheekie *et al.*[47]
8	*Nurse Education Today*	Pharmacology education: a theoretical framework of applied pharmacology and therapeutics	2003	Pharmacology education framework	Banning[48]
9	*Research in Social and Administrative Pharmacy*	Pharmacy emergency preparedness and response (PEPR): A proposed framework for expanding pharmacy professionals' roles and contributions to emergency preparedness and response during the COVID-19 pandemic and beyond	2020	PEPR framework	Aruru *et al.*[49]
10	*American Journal of Pharmaceutical Education*	Moving from injustice to equity: a time for the pharmacy profession to take action: the Rx-heart framework to address health equity and racism within pharmacy education	2021	Rx-HEART framework	Butler *et al.*[50]

Notes: There was no specified timespan for the publication search; all publications predating January 8, 2024, were eligible.
Abbreviation: ICAP: Interactive, constructive, active, and passive.

program could appropriately extend the overall training cycle.

Postgraduates should be instructed in the use of various bibliometric software (VOSviewer, CiteSpace, R, Biblioshiny, COOC, SATI, SPSS, etc.), which offer a wealth of practical tools to conduct bibliometrics research. Postgraduates' ability to evaluate and analyze the hot spots in drug research publications can be promoted after they grasp the basics of bibliometrics software. In summary, through the three-level division paradigm, we proposed the BAC framework. According to the above description, the BAC framework has a richer connotation and a more rigorous logic and, and has strong feasibility.

4.3. Significance

Through the above three-level division paradigm, the BAC framework has richer connotations for research training, a more rigorous logic, and strong practical feasibility. In the scientific research process, project initiation research is very important, and the ability to conduct such research must therefore be emphasized for the cultivation of independent scientific research abilities in postgraduate pharmaceutical students. The information obtained from bibliometric analysis can be used to explore new directions for scientific research and evaluate project feasibility. The quality of a bibliometric study may even determine the success or failure of the project. Therefore, the construction of the

Table 2. Tabular summary of the BAC framework

Level	Competency	Objective	Outcomes
A. Knowledge	Fundamental knowledge of bibliometrics	To gain basic understanding of bibliometric analysis	(1) Knowing what a research aim is and how to determine it; (2) Knowing what a retrieval strategy is and how to establish it; (3) Knowing what databases can be used for a literature survey and how to conduct a survey; (4) Knowing what software can be used for bibliometric analysis and how to process data using that software; (5) Knowing what methodologies can be used for data interpretation and how to implement them; (6) Knowing what the knowledge domain is and how to describe it.
B. Familiarization	Real-world material understanding	To independently understand the practical process of bibliometric analysis	(1) Understanding the reason for research aim determination in existing materials; (2) Understanding the logic of retrieval strategy establishment in existing materials; (3) Understanding literature survey procedures in existing materials; (4) Understanding software processing in existing materials; (5) Understanding the logic of data interpretation in existing materials; (6) Understanding the described knowledge domain in existing materials.
C. Mastering	Conducting bibliometric analysis	To independently conduct bibliometric analysis	(1) Mastering the ability to determine the research aim; (2) Mastering the ability to establish a retrieval strategy; (3) Mastering the ability to survey the literature; (4) Mastering the ability to use software for bibliometric analysis; (5) Mastering the ability to interpret bibliometric data; (6) Mastering the ability to describe the knowledge domain.

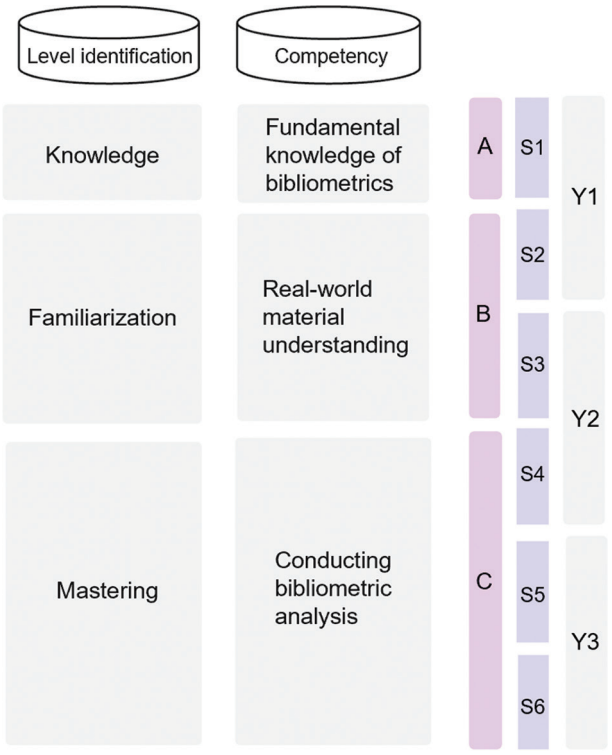

Figure 4. Graphical summary of the bibliometric analysis competencies framework
Abbreviations: S: Semester; Y: Year.

BAC framework will improve postgraduate pharmaceutical students' readiness to conduct independent scientific research in the future by optimizing their approach to project initiation.

Furthermore, the BAC framework meets the aforementioned developmental needs at the societal, governmental, and institutional levels. Society requires pharmacists with drug development capabilities to realize local governments' responses to public health emergencies along with the integrated development of industry, education, and research. In particular, the BAC framework complements the characteristics of contemporary postgraduate pharmaceutical training, which will help institutions enhance their uniqueness and academic reputation. Therefore, it is reasonable, scientific, and meaningful to implement BAC as a framework for planning, training, examining, and evaluating the independent scientific research abilities of postgraduate pharmaceutical students.

4.4. Implementation

Having proposed the BAC framework, the next consideration is its implementation. Based on the contents of Levels A-C as laid out in Table 2 and Figure 4, we propose the following implementation procedures:

(1) In Level A (knowledge), which corresponds to Semester 1 in a 3-year, six-semester postgraduate pharmaceutical program, lectures and individual asynchronous, facilitated pedagogy should be introduced. Postgraduate students will engage in bibliometrics courses, lectures, training, and

instructor-led cloud classroom learning and online discussions. Teaching Level A will primarily rely on a passive learning pedagogy.

(2) In Level B (familiarization), which corresponding to Semesters 2 and 3 of a 3-year, six-semester postgraduate pharmaceutical program, individual, asynchronous (non-facilitated), application-based learning exercises, and a team-based learning pedagogy should be introduced. Postgraduate students can join massive open online courses and study reference books independently. They will conduct independent research and group discussions according to their teachers' pre-determined questions on the six technical steps of bibliometric analysis and present their interpretations of these steps based on the materials they read in seminars. Level B can be achieved through active and constructive learning pedagogies.

(3) At Level C (mastering), which corresponds to Semesters 4 – 6 of a 3-year, six-semester postgraduate pharmaceutical program, problem-based learning, small group (non-facilitated), and simulation pedagogy should be introduced. Postgraduate students should be able to evaluate and critique the six technical steps of bibliometric analysis material provided in advance by the instructor, form small groups for self-directed learning, and use computers to conduct simulated bibliometric analyses on given research topics in communication sessions. Postgraduate students can achieve Level C through interactive learning (Table 3).[44]

The relevant elements are summarized in Table 3, based on the paradigm outlined in a previous study.[44]

Notably, Levels A and B in the above proposed implementation may need to be shortened for shorter programs; however, the training provided for Level C competencies should be retained, with an emphasis on achieving this level of competencies.

4.5. Assessment

Based on the BAC framework, the researchers developed an assessment system that focuses on the quantitative evaluation of the participating postgraduate students. The total proposed score was 100 points, including 10 points (10%) for a mentor interview, 40 points (40%) for a written report, and 50 points (50%) for a group presentation. A total score of <60 will be recorded as FAILED; 60 – 69 will be recorded as PASS; 70 – 79 will be recorded as MODERATE; 80 – 89 will be recorded as GOOD; 90 – 99 will be recorded as EXCELLENT; and 100 will be recorded as PERFECT. In this case, grades of PASS and above are considered as passing the BAC framework training, while students who receive FAILED grades require additional training.

The specific proposed grading rules are as follows:

(1) Mentor interviews. The main function of the interviews conducted by the instructor is to examine postgraduate students about their overall awareness of bibliometric analysis. The mentor determines what level (A – C) the postgraduate student's cognition has achieved, with 0 – 2 points for not achieving Level A, 3 – 4 points for achieving Level A without achieving Level B, 5 – 7 points for achieving Level B without achieving Level C, and 8 – 10 points for achieving Level C.

(2) Written report. The main content of the written report is a bibliometric analysis of a postgraduate student's

Table 3. Implementation of the BAC framework

Level code	Pedagogy	Examples	ICAP designation
A	Lectures	Bibliometrics courses, lectures, and training	Passive
	Individual asynchronous, facilitated learning	Instructor-led cloud classrooms and online discussions	Passive
B	Individual asynchronous, non-facilitated learning	Postgraduate students independently study MOOCs, literature, and references	Active
	Application-based learning exercises	Faculty members ask preset discussion questions on the six technical steps of bibliometric analysis, and postgraduate students conduct their own research and group discussions	Active
	Team-based learning	During seminars, postgraduate students develop interpretations of the bibliometric analysis material they have read based on the six technical steps	Constructive
C	Problem-based learning	Postgraduate students evaluate and critique the six technical steps of the bibliometric analysis material provided in advance by faculty members	Interactive
	Small groups (non-facilitated)	Postgraduate students form self-directed small groups	Interactive
	Simulations	After being given a research topic, postgraduate students use computers to conduct simulated bibliometric analyses	Interactive

Abbreviations: ICAP: Interactive, constructive, active, and passive; MOOCs: Massive open online courses.

self-selected research topic. Each technical step of the bibliometric analysis is scored according to both understanding and presentation, with each technical step being graded from 0 to 6 points, including 0 – 1 point for not reaching Level A, 2 – 3 points for reaching Level A without reaching Level B, 4 – 5 points for reaching Level B without reaching Level C, and 6 points for reaching Level C. In addition, the academic writing style and format of the academic report is scored from 0 to 4 points.

(3) Group presentation. The main form of the group presentation is a multimedia presentation report on a bibliometric analysis of a self-selected research topic by the postgraduate students in small groups, with each member of the group being scored equitably. The scoring module is synthesized from (1) and (2) above. First, the grader determines what level the group's cognition reaches from A to C. From here, 0 – 2 points are given for not reaching Level A, 3 – 4 points for reaching Level A without reaching Level B, 5 – 7 points for reaching Level B without reaching Level C, and 8 – 10 points for reaching Level C. Scoring is also conducted based on the understanding and presentation of the six technical steps, with 0 – 6 points given for each technical step. Here, 0 – 1 point is given for not reaching Level A, 2 – 3 points are given for reaching Level A without reaching Level B, 4 – 5 points are given for reaching Level B without reaching Level C, and 6 points are given for reaching Level C. In addition, the standardization of academic presentation style and format is given 0 – 4 points.

For simplicity, the above details are summarized in Table 4.

Table 4. Assessment system grading rules for the BAC framework

Total score	Section	Achievement	Score
100	Mentor interview (10)	<A	0 – 2
		>A, <B	3 – 4
		>B, <C	5 – 7
		>C	8 – 10
	Written report (40)	<A (for each technical procedure)	0 – 1
		>A, <B (for each technical procedure)	2 – 3
		>B, <C (for each technical procedure)	4 – 5
		>C (for each technical procedure)	6
		Normalization	0 – 4
	Group presentation (50)	<A (overall understanding)	0 – 2
		>A, <B (overall understanding)	3 – 4
		>B, <C (overall understanding)	5 – 7
		>C (overall understanding)	8 – 10
		<A (for each technical procedure)	0 – 1
		>A, <B (for each technical procedure)	2 – 3
		>B, <C (for each technical procedure)	4 – 5
		>C (for each technical procedure)	6
		Normalization	0 – 4

Based on the authors' teaching experience and the assessment system described above, a postgraduate student's assessment level can theoretically be assessed with a total score based on the contents of Table 4. For those who have not achieved Level A, the total score will be 0 – 24. For those who have achieved Level A but not Level B, the total score will be 30 – 52. For those who have achieved Level B but not Level C, the total score will be 50 – 83. Finally, for those who have achieved Level C, the total score will be 88 – 100. Based on these scores, those who have not achieved Level B will be categorized as FAILED and in need of supplemental training. Those who have achieved Level B but not Level C may be categorized as FAILED, PASSED, MODERATE, or GOOD, depending on the specific circumstances, and may need further training. Those who have reached Level C will be categorized as GOOD, EXCELLENT, or PERFECT, passing the training without further requirements.

4.6. Framework refinement

This framework is not a static system, and major and minor revisions should be conducted as necessary. First, a 3-year trial period should be conducted for this framework, tracking the BAC achievement of the subject postgraduate students from their 1st-year enrollment to their 3rd-year graduation. During this period, discussions should be held with postgraduate mentors to learn which parts the mentors feel need further optimization. The parts in need of optimization will also be determined based on the assessment results of the postgraduates. In addition, the international and domestic research reports on postgraduate pharmaceutical education should be continuously tracked, and the desirability of related frameworks should be appropriately evaluated and incorporated into optimization plans. The changes and trends in public health and the pharmaceutical industry should also be noted to improve the effectiveness of the BAC framework for all types of contingencies. Major and minor revisions will thus be made to the framework in several ways, as described above; the degree of concrete revisions will be determined and adjusted to ensure a constant and dynamic reform process.

4.7. Limitations

This framework has certain natural limitations. From a top-level design perspective, this framework is largely based on the teaching and research experience of the authors and their team; however, due to limited capacity and time, it was not possible to ensure the desirability of the established teaching and research experience. Thus, as there may be fallacies, the feasibility of the framework should be further validated.

From a practical implementation perspective, although this framework has theoretical basis and is guided by experience, it has not been piloted in a graduate pharmacy student population; therefore, its implementation requires further testing. The developed framework is expected to provide a useful reference for the reform of postgraduate pharmaceutical education in China. We plan to conduct an empirical study to test its efficacy and outcome.

The final limitation is in scope of application. The postgraduate training needs and ideas considered in designing this framework are mainly pertinent to China (and countries or regions with similar national conditions, especially some developing countries[51]) and may not be applicable to other countries or regions with different national conditions. Therefore, the potential of using the BAC framework in postgraduate pharmaceutical education in these other countries or regions should be assessed.

Despite these limitations, this framework still has significance for the reform and improvement of postgraduate pharmaceutical education. Through continuous real-world framework refinement, the potential problems in the top-level design and practical implementation can gradually be resolved. Furthermore, if there is a need to extend the framework to other countries and regions, the authors are eager to collaborate with interested institutions in those countries or regions. The core ideas of this framework can even help to establish the current situation of postgraduate pharmaceutical education in those countries or regions. Subsequently, the teaching and research experience from foreign institutions can be incorporated and an amended or new framework can be proposed.

5. Conclusion

Nowadays, there are three levels of demand for independent research skills in the pharmaceutical enterprise: these demands are from societies, from governments, and from institutions. This article proposed the integration of bibliometrics into Chinese postgraduate pharmaceutical program to establish a new framework as a theoretical guide for education reform. The BAC framework was designed by performing three levels of classification using a detailed assessment system. Institutions should carry out major and minor revisions to this framework to promote the reform of their postgraduate pharmaceutical programs. This framework could have great significance for postgraduate education through its continuous optimization using regular communication and real-time attention to developments in the domestic and international pharmaceutical industries. The continuous optimization and promotion of the framework will not only solve the problems of top-level design implementation and execution but also improve on China's current postgraduate pharmaceutical education system by enhancing the independent scientific research abilities of postgraduate pharmaceutical students, thus promoting the overall development of China's pharmaceutical industry.

Based on the findings, this study makes the following suggestions. First, it is necessary to incorporate professional bibliometrics training into the compulsory or elective courses of postgraduate students and combine theory with practice by studying real-world cases. Second, such training should be conducted by experts in bibliometrics, and advanced database resources and econometric tools should be introduced to facilitate students' econometrics learning and practice. Finally, promotional tools should be used to increase students' awareness about and interest in bibliometrics. Econometrics competitions or specific practical projects can also be conducted to strengthen students' practical skills.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

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Consent for publication

Not applicable.

Availability of data

Not applicable.

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ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

PERSPECTIVE ARTICLE

The transformative role of artificial intelligence in endoscopy

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Abstract

Artificial intelligence (AI) is transforming healthcare, with endoscopy emerging as a key area for its application. AI-driven tools are advancing gastrointestinal diagnostics by significantly improving the accuracy and efficiency of detecting conditions such as colorectal cancer, inflammatory bowel disease, and gastrointestinal bleeding. Notably, real-time AI-powered polyp detection has shown significant promise in reducing missed diagnoses, particularly for flat or subtle lesions. Furthermore, AI algorithms excel in lesion characterization, aiding in clinical decision-making and reducing the need for unnecessary biopsies. A major advantage of AI lies in its ability to mitigate variability in diagnostic performance, supporting less experienced endoscopists and contributing to standardized care across diverse clinical settings. Despite these advancements, challenges persist, including the need for large-scale validation of AI models, ensuring their generalizability across populations, addressing ethical and privacy concerns, and mitigating the risk of over-reliance on AI at the expense of human expertise. This perspective explores the transformative potential of AI in endoscopy, emphasizing the importance of thoughtful implementation, ethical considerations, and continued innovation to optimize its integration into clinical practice.

Keywords: Artificial intelligence; Endoscopy; Gastrointestinal diagnostics; Polyp detection; Colorectal cancer; Lesion characterization; Health-care technology

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1. Introduction

The integration of artificial intelligence (AI) into healthcare is revolutionizing various medical fields, with endoscopy emerging as one of the most promising areas for its application. Endoscopic procedures play a key role in the diagnosis and management of gastrointestinal diseases, including colorectal cancer, inflammatory bowel disease, and gastrointestinal bleeding. Despite advancements in endoscopic technology and techniques, these procedures remain heavily dependent on the skill, experience, and attentiveness of endoscopists. Challenges such as variability in diagnostic performance, missed diagnoses, and inefficiencies in care delivery persist, highlighting the need for innovative solutions.

AI offers a compelling solution to these challenges by augmenting human capabilities through advanced computational techniques, particularly machine learning and deep learning. This perspective explores the transformative potential of AI in endoscopy,

focusing on its role in enhancing diagnostic accuracy, standardizing care, addressing implementation challenges, and navigating ethical and practical concerns.

2. Enhancing polyp detection to reduce colorectal cancer mortality

Colorectal cancer is the second leading cause of cancer-related deaths worldwide, emphasizing the critical need for early detection and intervention. The adenoma detection rate (ADR) serves as a key quality indicator in colonoscopy, with higher ADRs directly correlating to reduced colorectal cancer mortality. Despite advancements, traditional polyp detection methods remain susceptible to human error, particularly for smaller, flat, or subtle lesions that are frequently overlooked.

AI-assisted endoscopy has demonstrated remarkable potential to overcome these limitations. Convolutional neural networks, a type of AI model trained on vast datasets of endoscopic images and videos, have shown the ability to identify polyps in real time with high sensitivity and specificity.[1,2] Clinical trials have reported significant improvements in ADR when AI tools are integrated into colonoscopy procedures.[3] These systems provide visual markers that highlight suspected polyps, enabling endoscopists to detect lesions that might otherwise go unnoticed.

The consistent improvement in polyp detection offered by AI is particularly valuable in high-risk populations and resource-limited settings, where access to experienced endoscopists may be constrained. By reducing missed diagnoses, AI has the potential to lower the incidence of interval colorectal cancers-those that arise between scheduled screenings.[4]

3. Advanced lesion characterization for personalized care

Beyond polyp detection, AI algorithms have demonstrated exceptional accuracy in lesion characterization, effectively distinguishing between benign and malignant growths. This capability stems from AI's ability to identify complex image patterns that are often imperceptible to the human eye. During real-time endoscopy, AI tools provide immediate feedback on lesion type, aiding clinicians in decisions such as whether to perform a biopsy or proceed with therapeutic intervention.[5]

This advanced diagnostic capacity carries several important implications for clinical practice. By reducing unnecessary biopsies and follow-up procedures, AI enhances the efficiency and cost-effectiveness of care. Patients benefit from timely, accurate diagnoses that facilitate earlier interventions and improved outcomes.[6] In addition, AI-driven lesion characterization simplifies treatment planning, allowing clinicians to adopt personalized approaches tailored to individual patient needs.

Emerging research also highlights the potential of AI to predict treatment outcomes based on lesion features. For example, certain AI models have demonstrated the ability to assess the likelihood of recurrence or progression in specific gastrointestinal conditions, further advancing precision medicine initiatives in gastroenterology.[7]

4. Reducing variability in endoscopic performance

One of the most significant challenges in endoscopy is the variability in diagnostic performance among practitioners. Factors such as experience level, fatigue, and workload can significantly influence the accuracy of lesion detection and characterization. Less experienced endoscopists, for example, often achieve lower ADRs than their more experienced counterparts, resulting in inconsistencies in patient care.[8]

AI systems offer a powerful solution to this issue by providing real-time decision support during procedures. Acting as a "second pair of eyes," AI reduces reliance on individual operator skill, ensuring a consistent standard of care. This is particularly valuable in regions with limited access to highly trained endoscopists, where AI can help democratize expertise and improve health-care equity.[9]

Moreover, AI tools serve as effective training resources for endoscopists. Real-time feedback from AI systems enables practitioners to identify areas for improvement and refine their techniques. Over time, this feedback loop can contribute to significant advancements in the overall quality of endoscopic care across health-care systems.

5. Challenges in AI implementation

5.1. Validation and generalizability

Despite its promise, the implementation of AI in endoscopy faces several challenges. A key concern is the validation and generalizability of AI systems across diverse clinical settings. AI models are typically trained on specific datasets that may not fully represent the variability in patient populations, imaging equipment, and clinical environments.[10] Ensuring the reliable performance of AI algorithms in real-world scenarios necessitates rigorous, large-scale, multicenter clinical trials.[11]

In addition, the performance of AI systems may vary based on factors such as image quality, bowel preparation, and the presence of comorbidities. Addressing these

variations is essential for building trust in AI tools and fostering their widespread adoption in clinical practice.[12]

5.2. Ethical and privacy concerns

The integration of AI into healthcare raises critical ethical and privacy concerns. AI systems rely on large volumes of patient data for training and validation, underscoring the importance of robust data security and privacy protection measures.[13] Health-care providers must implement robust measures to ensure that sensitive patient information is securely stored, managed, and shared in compliance with applicable regulations.

Moreover, ethical concerns arise from the potential over-reliance on AI systems. While AI can significantly enhance diagnostic accuracy, it is crucial that these tools complement rather than replace the expertise of human clinicians. Striking an appropriate balance between AI-driven decision support and human clinical judgment is vital for maintaining the quality and integrity of patient care.[14]

5.3. Regulatory and operational barriers

The regulatory approval processes for AI systems in healthcare are often complex and time-intensive, potential delaying their adoption in clinical practice. Furthermore, integrating AI tools into existing clinical workflows requires substantial investment in training healthcare professionals, upgrading infrastructure, and establishing comprehensive support systems. Overcoming these barriers will require close collaboration among AI developers, health-care providers, and regulatory bodies.

6. Future directions and opportunities

To fully realize the transformative potential of AI in endoscopy, ongoing research and innovation are essential. Future developments in this field may include the following:

(i) Integration with multimodal data: Combining AI analysis of endoscopic images with additional data sources, such as patient histories, genetic profiles, and biomarkers, to improve diagnostic accuracy and facilitate personalized treatment
(ii) Predictive analytics: Developing AI models capable of predicting patient outcomes, recurrence risks, and treatment responses, thereby supporting proactive care planning
(iii) Telemedicine applications: Leveraging AI to enable remote consultations and diagnostics, increasing access to high-quality gastrointestinal care in underserved regions
(iv) Continuous learning systems: Designing adaptive AI tools that can evolve and improve over time by incorporating new data, ensuring sustained relevance and optimal performance.

By addressing existing challenges and exploring these opportunities, AI has the potential to revolutionize endoscopy and transform gastrointestinal care.

7. Conclusion

AI represents a groundbreaking advancement in endoscopy, offering solutions to longstanding challenges in diagnostic accuracy, care standardization, and resource efficiency. By enhancing polyp detection, improving lesion characterization, and reducing variability in performance, AI offers the opportunity to elevate the quality of gastrointestinal diagnostics and care. However, fully realizing this potential will require thoughtful implementation strategies, rigorous validation processes, and a commitment to addressing ethical and practical concerns. As research progresses and innovations emerge, AI is poised to become an indispensable tool in endoscopic practice, driving substantial improvements in patient outcomes and the delivery of healthcare worldwide.

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ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

PERSPECTIVE ARTICLE

Repurposing anabolic agents in cancer cachexia: A perspective

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Abstract

Cancer cachexia kills millions of people worldwide every decade. Attenuating or mitigating this muscle-wasting condition associated with cancer could significantly reduce cancer-related deaths. The drug development pipeline takes 10 – 15 years to complete in the United States. During this period, hundreds of thousands to millions worldwide die of cancer cachexia. Medicines already approved by the United States Food and Drug Administration that cause significant muscle mass gains in healthy individuals could be used "off-label" or "repurposed" in individuals who do not have androgen receptor-responsive cancers. These agents are testosterone and anabolic steroids. In individuals with androgen receptor-responsive tumors, repurposing anabolic agents (e.g., beta-2 adrenergic agonist albuterol), which increases muscle mass through different mechanisms than the androgen receptor, is warranted. New research suggests that phenotyping for the androgen receptor must be conducted individually, even for the same cancer. The utilization of these two types of anabolic agents – those that use the androgen receptor and those that do not – in appropriate clinical trials in cancer cachexia is also warranted.

Keywords: Cancer cachexia; Testosterone; Anabolic steroids; Beta-2 adrenergic agonists; Prostate cancer; Breast cancer; Androgen receptor

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1. Cancer cachexia: Prevalence and mortality

Cancers are the second leading cause of death in the United States (US), surpassed only by cardiovascular diseases. Approximately 50% of cancer patients suffer from cachexia or disease-related muscle wasting.[1] This definition of cachexia proposed by Evans *et al.*[2] is a 5% weight loss in 12 months with an underlying disease, without fluid loss, along with a loss of muscle mass with or without fat loss, and at least three of the following five criteria: (i) Decreased muscle strength, (ii) fatigue, (iii) anorexia, (iv) low fat-free mass index, and/or (v) abnormal biochemistry results (elevated C-reactive protein and/or interleukin-6, anemia, and/or low serum albumin). Different types of cancer, disease stages, and patient characteristics[2] influence the prevalence of cachexia. Cancer cachexia, as defined in clinical practice, varies significantly due to a lack of consensus among physicians and researchers worldwide. Multiple groups[2-4] have differing definitions of cachexia. However, regardless of the definition of cachexia, the primary concern should be the high mortality rates associated with this condition. One challenge in the early and accurate diagnosis of cancer cachexia is the difficulty in obtaining an early diagnosis of cancer itself. Additional limitations include the need for specific patient data, such

as strength measurements, which take time and are not routinely conducted by oncology teams.

Approximately 80% of all cancer mortalities are associated with highly cachectic cancers, such as pancreatic and non-small cell lung carcinoma. The prevalence of cachexia is greater in males, those with a lower body mass index, and if sarcopenia is present. The use of chemotherapy will also increase the prevalence of cachexia. In Europe, the US, and Japan, the 1-year mortality rate is 20 – 80%.[1] Given that about 50% of cancer patients suffer from cachexia and the 20 – 80% 1-year mortality rate in these developed areas, 10 – 40% of all cancer patients die of cachexia or cancer-related muscle wasting. Our focus should be on the number of cancer patients dying rather than solely on the prevalence of cachexia. A rough calculation based on cancer incidence data in Europe (2018), the US (2022), and Japan (2022) estimates that cancer cachexia is responsible for 391,000 – 1,356,400 deaths in Europe,[5] 238,019 – 952,076 deaths in the US,[1] and 100,516 – 402,060 deaths in Japan.[1] Even if the 50% prevalence rate of cancer cachexia is a liberal estimate, there are still at least 50,000 deaths from cancer cachexia in each of Europe, the US, and Japan, and this is not a trivial problem. Therefore, cancer cachexia attenuation and mitigation could save hundreds of thousands of lives in each of these three geographical locations. This is an urgent issue, and the repurposing of anabolic agents could help stop or at least reduce these high mortality rates. It should be noted that the diagnosis of cachexia is less important than treating it with anabolic agents. In no circumstances would an increase in muscle mass be detrimental to a cancer patient's health, Grip strength is highly inversely related to mortality in the elderly.[6] Hence, it is proposed that treating all cancer patients with an appropriate anabolic agent would increase life expectancy and reduce mortality rates. Anabolic steroids or testosterone in non-androgen receptor-responsive tumors, and albuterol or other non-androgen receptor-binding anabolics in androgen receptor-responsive tumors, should be considered the standard of care. In simple terms: treat the cancer cachexia and discontinue treatment during remission.

Anabolic therapies can and have been combined with chemotherapy and radiotherapy in important clinical trials. This is crucial, as chemotherapy has been demonstrated to reduce muscle mass.[7]

2. Pharmacodynamics explaining higher response to pharmacological doses than physiological doses of a drug

The physiological and pharmacological molecular mechanisms that result in higher pharmacological response to androgens compared to physiological doses are explained by the Michaelis–Menten kinetics associated with androgen/receptor interactions, such as the substrate concentration that causes 1 - half maximum reaction velocity (K_m) and maximal reaction velocity (V_{max}). K_m is derived from the middle of a hyperbolic curve, in which the concentration at 50% V_{max} elicits a linear response but at half-maximal. Increasing the dosage increases the rate of receptor binding linearly until it reaches the maximal binding rate (i.e., the V_{max}), which is the plateau of the hyperbolic curve.[8]

3. Mechanism of action of androgens in skeletal muscle through the androgen receptor

Androgens bind to the androgen receptor in the cytoplasm of the cell; this complex then migrates into the nucleus and binds to DNA, inducing gene transcription in skeletal muscles. Two notable deviations from the strict ligand (steroid) specificity occur when dosages shift from physiological to pharmacological levels, as observed by Pihlajamaa *et al.*[9]: (i) Pharmacological dosages produce more pronounced effects than physiological dosages, and (ii) different steroid structures exhibit varying affinities for the androgen receptor, with some binding more effectively than others.[9] This prompts the research question: Can epigenetics affect the response to androgens? Through both extranuclear and nuclear mechanisms, androgens can activate or repress histone activity, thereby influencing protein signaling.[10]

4. Mechanisms of action of androgens in breast tissue, prostate tissue, and other tissues during metastatic cancer

Steroid-hormone-receptor interactions recruit signaling and/or scaffold proteins, stimulating flux through various pathways that promote cancer cell proliferation, survival, and migration for tissue invasion.[11] Several scaffold proteins[12] are as follows: (i) Steroid receptor co-activator-1 (Src)[13] is involved in androgen-induced prostate cancer cell proliferation; (ii) integrins are membrane linkers across cell membranes that interact with the cytoskeleton and extracellular matrix to induce metastasis and cell invasion;[14] (iii) filamin A is a cytoskeleton protein that regulates androgen responsiveness through the androgen receptor and downregulates androgen receptor function;[15] (iv) PI3K is a signaling kinase (phosphorylates) that is essential in insulin-signaling;[16] and (v) P130 Cas is involved in cell migration, apoptosis, and cell cycle control.[17] According to Giovannelli *et al.*,[11] the interaction of classical ligand-bound steroid receptors with scaffold and/or signaling proteins regulates cellular processes,

such as chromatin remodeling, cell cycle proliferation, and cell migration. In addition, the exclusion of nuclear steroid receptors and relegation to the cytoplasm may be controlled by the interaction of these receptors and signaling and/or scaffold proteins. Androgens can activate or repress histone activity through extranuclear and nuclear mechanisms, thereby influencing protein signaling and exerting epigenetic effects.[10] Androgens exert similar effects in androgen receptor-positive tissues (prostate, breast, and skeletal muscle); these effects are generally anabolic and/or proliferative responses.

Another factor that affects the anabolic response to androgens is the tumor microenvironment. For example, tumor necrosis factor-alpha in the tumor microenvironment is a negative regulator of protein metabolism[18] that promotes degradation and reduces protein synthesis in muscles, suggesting competing influences on the anabolic response to androgens.

Isomers with the same chemical formula but different atomic arrangements can affect receptor binding due to altered specificity. For example, glucose binds to its receptor with much more binding affinity than fructose. Despite having the same chemical formula ($C_6H_{12}O_6$), the fructose isomer has a lower binding affinity toward glucose receptors.

5. Repurposing of anabolic agents

If the high mortality rates are not compelling enough, the lengthy time required to bring a new drug to market, the immense cost of this process, and the countless lives lost during this period should be truly eye-opening. The entire process from drug discovery to full Food and Drug Administration (FDA) approval, inclusive of pre-clinical and clinical trials, takes approximately 10 – 15 years. At a moderate mortality rate of 500,000/year, a 10-year FDA approval process would result in 5 million deaths in the US. While repurposing anabolic agents may not save all lives, bodybuilders allegedly taking these agents may gain 5 – 10 kg of muscle with resistance training[19] in a short period. Bhasin *et al.*[19] reported that healthy men receiving 600 mg/week of testosterone enanthate, combined with resistance training, gained 6.1 kg of fat-free mass in 10 weeks. This accrual of skeletal muscle tissue in cancer patients would allow time for other oncology practices, such as chemotherapy, radiotherapy, surgery, and immunotherapy, to take effect and eliminate tumor-derived cancer. Cancer patients who die from cachexia are often believed to succumb to respiratory muscle atrophy and failure. Considering the time, cost, and lives lost, it is imperative to repurpose anabolic agents, such as testosterone and steroids. In the first clinical trial investigating the effects of anabolic agents on cancer cachexia, Wright *et al.*[20] reported that the administration of 100 mg of testosterone to cervical and head and neck cancer patients undergoing chemotherapy and chemoradiation resulted in a 3.2% increase in muscle mass in the testosterone group and a 3.3% decline in the placebo group after 7 weeks of administration. There was no improvement in muscle strength, physical performance, and survival, but there was an improvement in the quality of life. Limitations of this study include the low dose of testosterone administered in a life-threatening situation and the relatively short duration of administration. Given the high risk of mortality in these patients, this should encourage physicians and scientists to adopt more aggressive approaches. The likelihood of liver problems is low with injectable testosterone or anabolic steroids. In addition, the use of anabolic steroids (e.g., nandrolone decanoate) in high doses would be safer than high doses of testosterone due to the relative androgenicity between the two agents. The higher dose of testosterone (600 mg/week) used in normal non-cancer patients by Bhasin *et al.*[19] resulted in a 6.1 kg increase in lean body mass in healthy individuals. Moreover, the typical testosterone cycle for a powerlifter involves 1 – 2 g/week, along with 50 – 100 mg/day of Dianabol or Anadrol for 8 – 16 weeks. These strength athletes live healthily and do not experience immediate mortality after completing the cycle.[21]

The anabolic response would be greater in a cancer patient depleted of skeletal muscle compared to healthy controls, due to a potential ceiling effect. Based on my understanding of floor and ceiling effects, anabolic agents should be used at all stages of cancer. Early use could prevent a significant decline in muscle mass, and theoretically, the more negative the nitrogen balance, the greater the corrective effect anabolic agents could have. Notably, high-energy (kcals) and protein/amino-acid intake, either enterally or parenterally, should accompany high-dose testosterone or anabolic steroid administration in such a situation.

6. Androgen sensitivity or androgen receptor presence in different tumor types

The presence of androgen receptors in tumors suggests that these tumors will grow when stimulated by androgens. Therefore, for certain cancers, androgen receptor stimulation using testosterone, anabolic steroids, and other androgens is not an option. One alternative is to treat androgen-sensitive tumors with anabolic agents that do not stimulate the androgen receptor.

According to Viehweger *et al.*[22] androgen receptor-positivity, measured through immunohistochemistry, is similar in testicular and ovarian stromal tumors as it is in

prostate tumors. Moreover, breast, renal, and urothelial cancers have high androgen receptor expression. However, androgen receptor expression varies across different tumor types, with an approximate expression of 25 – 75% in breast cancer.

The expression of the androgen receptor is not homogeneous for all tumors of the same type. Some patients are androgen receptor-positive, whereas others are not for a given tumor type. Breast cancer can be androgen receptor-positive or androgen receptor-negative, suggesting that the same type of cancer may have tumors that are proliferative to androgens, whereas others are not responsive. Individual testing of biopsy samples from the tumor with immunohistochemistry could help with this approach. For androgen receptor-negative tumors, androgens may be used. In contrast, for androgen receptor-positive tumors, non-androgenic anabolics such as albuterol could be more appropriate.

7. Discussion

Cancer cachexia affects many cancer patients, and developing new drugs for this condition is a long and costly process, with an estimated 5 million cancer patients potentially dying in the meantime. Repurposing already approved anabolic agents, such as testosterone and anabolic steroids, could substantially reduce the mortality rate of cancer cachexia.

One potential treatment that required rigorous clinical trials in androgen receptor-positive cancers is albuterol. The rationale for using albuterol in these cancers is that androgens can stimulate tumor proliferation. Uc *et al*.[23] previously reported in Parkinson's patients that albuterol was anabolic to skeletal muscle. In a previous study,[23] a 5.3% increase in lower limb muscle mass (quadriceps femoris) and a 9.5% increase in whole body lean body mass were observed after 14 weeks of albuterol administration (16 mg/day). Lambert *et al*. (unpublished data) observed a 90% increase in skeletal muscle protein synthesis (in the vastus lateralis skeletal muscle) as a result of 16 mg/day albuterol administration for 10 days in healthy elderly individuals. Future clinical trials involving albuterol in androgen receptor-positive cancer patients are warranted to help attenuate and mitigate death from cancer cachexia. The mechanism of action of albuterol is likely due to the increase in cAMP intracellularly through stimulation of the beta-2 adrenergic receptor, thereby inducing the phosphorylation of various kinases within the skeletal muscle cell.[24] The exact mechanism by which albuterol stimulates skeletal muscle protein synthesis remains unknown, warranting further studies. Other signaling pathways for beta-2 adrenergic agonists include tyrosine kinases, which can phosphorylate the beta-2 adrenergic receptor at multiple sites, deactivating it through insulin. Insulin-like growth factor (IGF-1) can also phosphorylate and deactivate the receptor. In addition, eukaryotic initiation factor 2B activates the beta-2 adrenergic receptor, whereas arrestin is involved in decreasing receptor activation and signaling. There may be other mechanisms of action for beta-2 adrenergic agonists in the skeletal muscle and interactions with other pathways, but these have yet to be elucidated. A book chapter published nearly two decades ago[25] suggested that IGF-II could mediate beta-2 adrenergic agonist activation in skeletal muscle.

8. Conclusion

Testosterone, anabolic steroids, and albuterol are promising options to reduce the loss of ~5 million lives over the next 10 – 15 years. These agents, though not extensively studied in cancer cachexia, offer hope if used properly and for sufficient duration.

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Conflict of interest

The author declares he has no competing interests.

Author contributions

This is a single-authored article.

Ethics approval and consent to participate

Approval for Albuterol study was obtained from the local ethics commitee.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

ORIGINAL RESEARCH ARTICLE

Brain glutamate level after treatment with N-acetylcysteine in obsessive-compulsive disorder patients: A randomized trial

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Abstract

Selective serotonin reuptake inhibitors (SSRIs) are routinely used to treat patients with obsessive–compulsive disorder (OCD); however, 40 – 60% of patients with OCD do not respond to SSRIs. Glutamate dysfunction may play a key role in OCD pathogenesis. N-acetylcysteine (NAC), a glutamate-modulating drug, targets the glutamatergic system. This study aimed to assess whether the addition of NAC reduces the severity of OCD symptoms in patients with SSRI-treated moderate-to-severe OCD. A total of 60 patients with OCD were diagnosed according to the DSM-5 criteria, and severity of the symptoms was assessed using the Yale–Brown obsessive–compulsive scale (Y-BOCS). Patients were administered 2,400 mg/day of SSRIs plus placebo (placebo arm) or 2,400 mg/day (NAC arm) of SSRIs plus NAC for 10 weeks. Serum alanine aminotransferase, aspartate aminotransferase, creatinine, and electrocardiogram were monitored to evaluate the safety of NAC. The Y-BOCS score was not significantly different between the two arms at baseline; however, it was significantly different between the two arms after 4 ($P = 0.03$) and 10 ($P = 0.00$) weeks. The NAC arm had a reduction of 8.4 (25.51 – 17.15) points compared with 1.42 (25.07 – 23.65) points for the placebo arm from baseline to 10 weeks. NAC was well-tolerated and caused mild gastrointestinal adverse events. Thus, NAC is an effective glutamate-modulating drug as and can be used as an augmentation therapy with standard treatment in patients with moderate-to-severe OCD.

Keywords: Glutamate; N-acetylcysteine; Obsessive–compulsive disorder; Yale–Brown obsessive–compulsive scale

1. Introduction

According to the American Psychiatric Association (2013), obsessive–compulsive disorder (OCD) is a chronic and debilitating mental illness, characterized by repetitive behaviors or mental activities (compulsions) and frequent and intrusive thoughts, images, or urges (obsessions).[1] The lifetime prevalence of OCD in the United States is 1 – 3%.[2,3] According to Algin *et al.* (2016), 70% of adult patients in Bangladesh have moderate-to-severe OCD.[4] OCD and other anxiety disorders are the sixth most common causes of non-fatal health loss worldwide.[5] OCD is predominantly related to disability and an increased risk of early death.[6,7] The American Psychiatric Association included OCD as a separate category in the Diagnostic and Statistical Manual of Mental Disorders (DSM)version 5,[8] replacing its previous classification as an anxiety disorder. Notably, patients with OCD also experience impairments in cognitive skills, such as attention, memory, decision-making, and inhibitory control.[9]

Although several mechanisms, including oxidative stress, infection, inflammation, autoimmune processes, and disorganized neurotransmission, have been proposed as underlying causes, the specific etiology of OCD remains unknown.[10,11] An abnormality in the serotonin pathway is considerably the primary cause of its development, due to its selective sensitivity to serotonergic medications. The involvement of glutamate, dopamine, and other neurochemicals has been reported.[12] Serotonergic antidepressants (selective serotonin reuptake inhibitors [SSRIs]) are recommended as first-line therapy by most clinical practice guidelines.[13,14] However, first-line treatments and other augmentation measures do not sufficiently treat 40 – 60%of patients with OCD.[15-18] In addition, psychological treatments take time and are not affordable for some patients. Moreover, full functional recovery is relatively rarely achieved, and high doses of SSRIs prescribed for patients with OCD involve side effects, such as sleepiness, insomnia, and sexual dysfunction.[19-21] These factors often demand the use of additional adjunctive treatments.[22-25] Numerous strategies have been attempted to improve treatment response, from adding cognitive behavioral therapy[26] to elevating serotonergic action by increasing the dosage, combining SSRIs, or using the intravenous route.[27,28] In addition, other medications have been attempted, including neuroleptics, anticonvulsants, opioids, and neurosurgical techniques.[29]

Numerous glutamate-modulating medications, such as lamotrigine, riluzole, and memantine, have been evaluated as monotherapy or augmentation therapies for OCD treatment.[30] Reportedly, the pathophysiology of OCD may be influenced by an impaired glutamatergic system. This system has recently gained interest as a possible treatment target for OCD.[10,30,31] Glutamatergic drugs can function as ion-channel modulators, coagonists, reuptake inhibitors, and receptor antagonists. Changes in glutamate levels could influence the onset and persistence of OCD symptoms.[32] Glutamate is the primary excitatory neurotransmitter of the cortico-striatal-thalamo-cortical (CSTC) circuit and is essential for the proper development of the CSTC circuitry, neuronal transmission, plasticity, and general brain function.[33] Increasing evidence correlates the pathophysiology of OCD to disruptions in glutamate neurotransmission, in addition to the serotonin transporter system.[34-38] Reportedly, the cerebrospinal fluid of patients with OCD has a substantially higher glutamate level than that of healthy controls.[37,39] High glutamate levels are associated with oxidative stress and excitotoxicity and can be related to the intensity of OCD symptoms.[40,41]

Due to the importance of glutamate, new treatment approaches have been developed. The glutamatergic system is targeted and modulated by N-acetylcysteine (NAC), a derivative of the amino acid cysteine. NAC contributes to cysteine, which is essential for glutathione (GSH) production. Although cysteine is not used in this process, it is transported across the blood–brain barrier by sodium-dependent mechanisms. After entering the central nervous system, it undergoes oxidation to produce cysteine.[42] Through a cystine–glutamate antiporter, astrocytes exchange cysteine for glutamate, which is released into the extracellular space. This activation of inhibitory metabotropic glutamate receptors mGLuR2/3 on glutamatergic nerve terminals reduces the amount of synaptically released glutamate.[43,44]

Furthermore, GSH enhances the NMDA receptor response to glutamate in the brain. Consequently, altered neuronal GSH levels may directly affect glutamatergic function, in addition to changing the amount of accessible glutamate.[45] NAC may also have biological effects on the brain, which are potentially related to OCD, such as reducing the production of inflammatory cytokines and modifying dopamine release. These characteristics would increase growth factors, such as brain-derived neurotrophic factor, and regulation of neuronal cell death through the expression of B-cell lymphoma 2, in addition to oxidative stress reduction and glutamatergic balance restoration.[42,44]

To the best of our knowledge, only seven randomized controlled trials have examined the potential effectiveness of NAC as an augmentation treatment for OCD, four of which revealed that taking 2,000 – 3,000 mg/day significantly decreased the Yale–Brown Obsessive–Compulsive Scale (Y-BOCS) scores.[46-52] In a 12-week

study, Afshar *et al.* (n = 48) found that the total Y-BOCS score of the NAC group decreased by 10.9 points, whereas that of the placebo group decreased by 5.7 points (P = 0.003).[52] Paydary *et al.* conducted a 10-week study in which 44 patients with OCD who had stopped taking psychiatric medicines 6 weeks earlier were randomized to receive 200 mg/daily fluvoxamine + placebo or 200 mg/daily fluvoxamine plus 2 g/daily of NAC. According to Paydary *et al.* (2016), the NAC augmentation group experienced a higher slope of treatment response than the placebo augmentation group (P = 0.012).[50] In contrast, Sarris *et al.* (n = 44) and Costa *et al.* (n = 40) reported that NAC is not beneficial on the overall Y-BOCS scores by the study endpoint, despite having longer trial lengths and greater NAC dosages (16-week trials with a 3 g/day dosage).[49,51] Sarris *et al.* found no table improvements favoring NAC on the "compulsions" subscale of Y-BOCS at week12. Compared with placebo, another study did not find evidence that the use of NAC as an augmentation therapy mitigated the OCD symptoms.[46] In a case series and case report of patients with OCD treated with SSRIs and NAC, the Y-BOCS score decreased, and symptoms improved considerably.[53,54] Further studies with larger sample sizes are needed considering the conflicting findings from previous RCTs of NAC for OCD. In addition, long trial periods might be necessary to identify the possible advantages of NAC.[55] These results suggest that NAC can effectively supplement the existing first-line treatments for OCD. NAC has been proposed as a practical augmentation due to its low cost and excellent tolerability, particularly in the refractory subset of patients with OCD, in which insufficient responses are noted and few alternatives are available.[56] Even at very high dosages, NAC was reportedly safe.[42] Thus, this randomized, double-blind, placebo-controlled study aimed to assess the safety and effectiveness of NAC as a glutamate-modulating drug in patients with moderate-to-severe OCD who were using SSRIs.

2. Materials and methods

2.1. Study design and participants

Patients with moderate-to-severe OCD were recruited from the outpatient departments of Bangabandhu Sheikh Mujib Medical University (BSMMU) and Combined Military Hospital (CMH), Dhaka, and participated in this randomized, double-blind, placebo-controlled, 10-week multi-center trial from March 2020 to January 2022. A total of 60 patients were recruited, with 30 patients in the intervention and 30 in the control arms. Patients in the two arms randomly received SSRIs plus placebo (2,400 mg/day) and SSRIs plus NAC (2,400 mg/day). The dose of NAC at week 1 was 600 mg/day, which was increased to 1,200 mg/day in two divided doses at week 2. The daily dose for NAC from weeks 3 to 10 was 2,400 mg/day (1,200 mg bd). Two sets of random numbers were assigned as patient code numbers. One set was designated as the intervention arm, whereas the other set was the control arm.

Eligible patients were newly diagnosed patients aged ≥18 years. Only patients with SSRIs (fluoxetine, sertraline, or fluvoxamine)-treated OCD who met the DSM-5 criteria and had a Y-BOCS score of ≥16 (moderate-to-severe) were enrolled. The Y-BOCS scale is considered the gold-standard clinician-rated scale with subtotals for the degree of obsessions and compulsions. Each item is evaluated from 0 (no symptoms) to 4 (severe symptoms). The total score of 0 – 40, with separate subscales for obsessions and compulsions.[57] Patients who had a history of any other psychiatric disorders and/or medical conditions before exposure to NAC within the last 2 months and those who did not provide consent were excluded from the study.

2.2. Outcomes

The primary outcome measure was the reduction of OCD severity from baseline to after 10 weeks measured by Y-BOCS-10. Secondary outcome measures were the safety and tolerability evaluations of NAC by serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine, and electrocardiography (ECG) for evaluating the liver, kidney, and heart functions at baseline and after 10 weeks that NAC was given at a high dose.

2.3. Study procedure

The enrollment and recruitment of patients were performed according to the principle of Consolidated Standards of Reporting Trials. NAC, 600 mg effervescent tablets, and placebo tablets were purchased from the Unimed and Unihealth Manufacturers (Dhaka, Bangladesh) at the original market price.

2.4. Randomization and masking

Randomization was performed by online graph pad software using a computer. After providing the necessary inputs (sample size and number sets), the software generates two distinct sets of random numbers. The calculated number of samples was randomly allocated in two arms. Subsequently, the set of code numbers that belong to the intervention arm was written as patient code numbers on the packages containing NAC tablets. Conversely, the set belonging to the control arm was designated as patient code numbers on the packages containing placebo tablets. The entire randomization process was performed by people unrelated to this research. Thus, the participants, outcome assessor, and analyst were unaware of the received treatment.

2.5. Intervention and assessments

A competent clinical psychologist diagnosed patients with OCD were diagnosed based on the DSM-5 criteria. After obtaining informed written consent, 60 patients were enrolled. Next, address, phone number, and demographics were collected using a prepared data sheet. However, three patients from the placebo arm and three from the NAC arm dropped out of the trial. Therefore, 27 and 27 patients from the placebo and NAC arms completed the trial. Therefore, the intention-to-treat protocol included 60, and the per-protocol treatment included 54 patients, who were available to complete the study. At baseline, the patients were assessed by Y-BOCS. Subsequently, patients were given NAC and placebo boxes. The study included three visits: one baseline visit and two follow-up visits after 4 and 10 weeks with a visit window of ±4 days. Patients were visited to assess their response to treatment using the Y-BOCS after 4 and 10 weeks.

2.6. Statistical analysis

All data were presented as a mean ± standard deviation, and analysis was performed using Microsoft Excel. For quantitative and qualitative variables, an unpaired t-test and Chi-squared (χ^2) test were used, respectively. $P < 0.05$ was considered significant.

2.7. Patient assessment tools

DSM-5 and Y-BOCS were used as patient assessment tools.

3. Results

As shown in Figure 1, 60 patients were enrolled based on the eligibility criteria. Patients were randomized into two arms to receive SSRIs plus placebo and SSRIs plus NAC.

Table 1 shows the demographic characteristics of the patients (n = 54) at baseline. No significant difference was found in age and sex between the two arms (P = 0.10 and 0.61).

Table 2 shows the Y-BOCS score of the patients at baseline and after 4 and 10 weeks. At baseline, the Y-BOCS scores in the placebo (25.07 ± 2.21) and NAC (25.51 ± 3.05) arms were not significantly different (P = 0.55). Toward the end of this trial, the Y-BOCS score reduction in the NAC arm was significantly different from that in the placebo arm ($P < 0.05$). The Y-BOCS score decreased from 25.51 ± 3.05 to 17.15 ± 5.20 in the NAC arm (Figure 2). The score in the placebo arm changed from 25.07 ± 2.21 to 23.65 ± 2.20. By the end of week 4, the first sign of an improving effect of NAC on the Y-BOCS score was noted (P = 0.03, Figure 2). Of 27 patients in the NAC arm, 20 (74.06%) reported a complete clinical response. In the placebo arm, this ratio was 7.40% (1/27).

Table 3 shows the safety parameters between the placebo and NAC arms at baseline and after 10 weeks of

Table 1. Demographic characteristics of patients (n=54) during enrollment

Variables	Placebo+SSRI (n=27) (Mean±SD)	NAC+SSRI (n=27) (Mean±SD)	P
Age in years	29.00±9.18 Range (18 – 48)	26.83±5.53 Range (18 – 42)	0.10[x]
Sex			
Male	12/27 (44.44%)	11/27 (40.74%)	
Female	15/27 (55.55%)	16/27 (59.25%)	0.61[y]

Note: [x]Unpaired t-test; [y]Chi-squared (χ^2) test
Abbreviations: NAC, N-acetylcysteine; SD, standard deviation; SSRI: Selective serotonin reuptake inhibitor.

Table 2. Comparison of the Y-BOCS scores between the control and intervention arms (at baseline and after 4 and 10 weeks of treatment)

Variable	Placebo+SSRI (n=27) Mean±SD	NAC+SSRI (n=27) Mean±SD	P[x]
At baseline	25.07±2.21	25.51±3.05	0.55
After 4 weeks	24.15±3.66	21.96±3.53	0.03
After 10 weeks	23.65±2.20	17.15±5.20	0.00

Note: [x]Unpaired t-test.
Abbreviations: NAC: N-acetyl cysteine; SSRI: Selective serotonin reuptake inhibitor; Y-BOCS: Yale–Brown obsessive scale.

Table 3. Safety parameters between the placebo and NAC arms (at baseline and after 10 weeks of treatment)

Variables	Placebo+SSRI (n=27) Mean±SD	NAC+SSRI (n=27) Mean±SD	P[x]
Serum ALT (U/L)			
At baseline	27.50±18.17	26.96±14.38	0.90
After 10 weeks	28.81±18.99	26.18±13.25	0.56
Serum AST (U/L)			
Atbaseline	26.84±13.74	24.41±8.32	0.44
After 10 weeks	28.23±1.98	23.81±6.47	0.15
Serum creatinine (mg/dL)			
At baseline	0.79±0.19	0.79±0.16	0.85
After 10 weeks	0.80±0.17	0.78±0.13	0.48
ECG			
Normal	5	12	0.07[z]
Abnormal	0	1	

Note: [x]Unpaired t-test; [z]Chi-squared (χ^2) test.
Abbreviations: ALT: Alanine transaminase; AST: Aspartate aminotransferase; ECG: electrocardiogram; NAC: N-acetylcysteine; SD: Standard deviation; SSRI: Selective serotonin reuptake inhibitor.

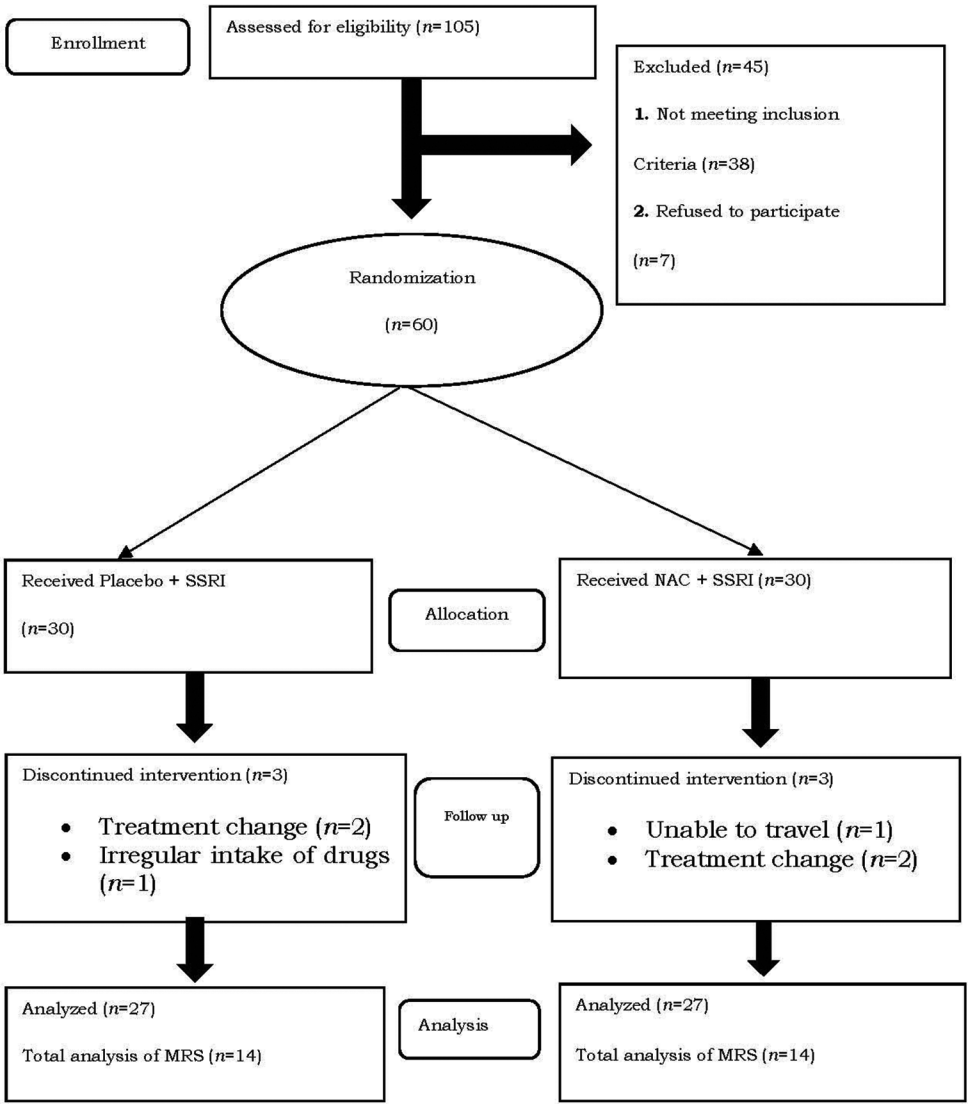

Figure 1. CONSORT Flowchart
Abbreviation: CONSORT: Consolidated Standards of Reporting Trials.

treatment. Liver aminotransferases and serum creatinine did not increase significantly at the end of the intervention period in any of the two arms, and no significant difference was recorded between the arms (P = 0.56, 0.15, and 0.48 for ALT, AST, and serum creatinine, respectively). Furthermore, no clinically significant elevations have been reported. No abnormalities were detected on the ECG.

Table 4 shows the number of adverse effects in the NAC and placebo arms during the treatment. The difference between the two arms was not significant after 10 weeks of treatment (P = 0.89). Adverse effects in the placebo arm were constipation (n = 2), headache (n = 2), insomnia (n = 2), dizziness (n = 1), diarrhea (n = 1), and nausea (n = 1), and those in the NAC arm were nausea (n = 2), diarrhea (n = 2), constipation (n = 1), headache (n = 1), dizziness (n = 1), and insomnia(n=1). All adverse effects were mild and did not require treatment discontinuation.

4. Discussion

This randomized, double-blind, placebo-controlled trial evaluated the safety and efficacy of NAC as augmentation therapy with SSRIs in patients with moderate-to-severe OCD. Both arms were followed up for 10 weeks, and their treatment response was evaluated using Y-BOCS. The addition of NAC to an SSRI medication was more effective than a placebo on lowering OCD severity. The results showed a significant reduction in the total Y-BOCS scores

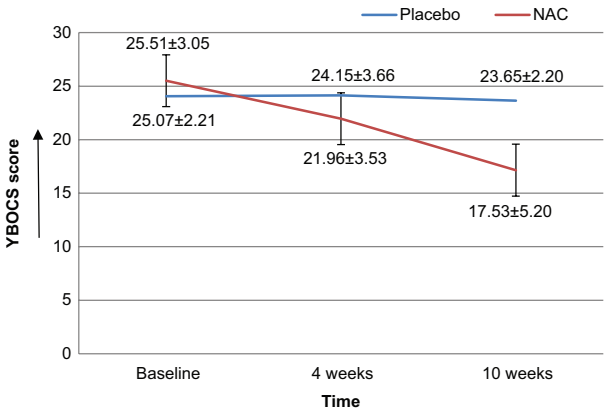

Figure 2. Changes in the Y-BOCS score over time in the placebo and NAC arms
Abbreviations: Y-BOCS: Yale–Brown obsessive–compulsive scale; NAC: N-acetylcysteine.

Table 4. Adverse effects in the placebo and NAC arms during treatment

Adverse effect	Placebo+SSRI (*n*=27) (%)	NAC+SSRI (*n*=27) (%)	*P*[x]
Constipation	2 (7.69)	1 (3.84)	0.89
Dizziness	1 (3.84)	1 (3.84)	
Headache	2 (7.69)	1 (3.84)	
Insomnia	2 (7.69)	1 (3.84)	
Diarrhea	1 (3.84)	2 (7.69)	
Nausea	1 (3.84)	2 (7.69)	

Note: [x]Chi-square (χ^2) test was done.
Abbreviations: NAC: N-acetylcysteine; SSRI: Selective serotonin reuptake inhibitor.

of patients in the NAC arm compared with those in the placebo arm during the trial.

Recently, the role of glutamate signaling pathways in the pathophysiology of OCD has been recognized.[37,58] Consequently, the use of medicines that modulate glutamate levels has been proposed as a new approach to treating OCD. Despite the lack of studies in this area, two studies explored the use of glutamate-modulating medications as a monotherapy or augmentation strategy in the OCDtreatment.[10] Considering the neurotransmission-regulating qualities of NAC[59] and the involvement of oxidative stress in psychological illnesses,[60] NAC was suggested as a psychoactive substance and was studied for various psychological disorders.[44] NAC targets the glutamatergic system and improves the general condition of patients with OCD. Despite the limited studies on its effect on OCD symptoms, NAC is reasonably available and safe.[61,62] To our understanding, NAC affects the severity of OCD symptoms in three mechanisms. First, it can modulate glutamate. NAC can cross the blood–brain barrier and enhance extraneuronal cysteine levels. An antiporter predominantly found on glial cells facilitates the reverse transfer of glutamate and cysteine. This will increase the presence of free glutamate in the extracellular extrasynaptic region. When inhibitory metabotropic glutamate receptors (group 2) on nerve terminals are stimulated, extrasynaptic glutamate can decrease the release of synaptic glutamate.[59] Second, NAC acts in general antioxidant activities via metabolic assistance to GSH formation by supplying an extra concentration of cysteine.[63] Increased GSH availability reduces oxidative and nitrosative stress, and consequently cell damage, by scavenging toxic by-products of glutamatergic and dopamine metabolism.[64] NAC improves the removal of synaptic glutamate in the central nervous system by sparing glial cells from glutamate toxicity.[65] The anti-inflammatory effects of NAC are possibly the other mechanism underlying its benefits for OCD. In addition to a few studies that demonstrated the ability of NAC to reduce some inflammatory cytokines, such as interleukin-6 and tumor necrosis factor-α,[66] this idea was derived from the underlying role of inflammatory cytokines in some psychiatric disorders, including OCD.[44]

Herein, after unblinding, no significant differences were observed in age or sex between the placebo and NAC arms. After 4 weeks, daily administration of NAC resulted in a significant reduction of the Y-BOCS score in the NAC arm; however, no reduction was noted in the placebo arm, and a significant difference was noted between the placebo and NAC arms. Considerably, the improvement attributed to the addition of NAC is significant after 4 weeks. After 10 weeks, this reduction was even greater, with an average reduction of 8.4 points from baseline, and the difference between the placebo and NAC arms was significant. Herein, NAC was more effective than placebo in lowering the severity of OCD symptoms. Furthermore, after 10 weeks of follow-up, over half of the patients in the NAC arm demonstrated full clinical response, which was more than that of the placebo arm. This finding was consistent with the results of prior clinical trials and case reports on the efficacy of NAC.[48,50,52,54] This may be due to the same NAC doses and study duration. This finding was inconsistent with those of other studies,[46,49,51] potentially due to the longer OCD duration, comorbidities, and treatment-resistant OCD. Previous studies involving patients with substance use disorders, addiction, and depression have produced conflicting results regarding the effectiveness of NAC as augmentation therapy.[67] Herein, the findings did not align with those of another case series,[68] which may be due to the presence of refractory OCD in the case series.

Furthermore, this study analyzed the adverse effects of NAC on OCD symptoms. NAC was well-tolerated. No

significant adverse effects were recorded, and the rates of various adverse effects were not different between the NAC and placebo arms. Compared with previous studies examining the effectiveness of NAC in OCD, this study had a larger sample size, double-blind, randomized, placebo-controlled design, and clearly specified inclusion/exclusion criteria.

5. Limitations

NAC was administered at the highest dosage starting in week 3. Thus, to effectively treat OCD symptoms, long durations or high dosages are needed. A small sample was analyzed; thus, results with a larger sample size might be trustworthy and accurate. Furthermore, whether the effects of NAC are durable or if the symptoms would recur if NAC was stopped is unclear. Finally, the use of magnetic resonance spectroscopy, a neuroimaging approach, to evaluate glutamate activity and other important biomarkers or pharmacodynamic pathways in the brain would have been adequate.

Considerably, this study does not support the suggestion that NAC should be used regularly as an adjuvant drug to treat adult OCD symptoms. Therefore, the effect of NAC on OCD must be examined. Future studies should examine how NAC works in conjunction with other drugs to treat OCD.

6. Conclusion

NAC is safe and effective to be used as augmentation therapy with standard treatment (SSRIs) in adult patients with moderate-to-severe OCD, results in earlier and greater reduction of OCD symptoms, and reduces the burden of this distressing illness.

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Conflict of interest

The authors declared that they have no competing interests.

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Ethics approval and consent to participate

The Helsinki Declaration and subsequent changes were followed in conducting the study. The research protocol was approved by the institutional review board (IRB) of Bangabandhu Sheikh Mujib Medical University, Dhaka, BD, on March 15, 2021 (approval number BSMMU/2021/2300). This clinical trial was registered on ClinicalTrials.gov with the trial ID number NCT04904952. Every patient provided written, informed consent, and every patient was informed about the intervention and the study objectives. They were also informed that there was the least possible chance of any harm to the patient by inclusion in this study, and if any complication arose due to the intervention, they were offered free treatment for that complication. Patients were informed that they could participate in this study of their own free will and that they were free to refuse to participate or to withdraw at any time without compromising their medical care. Patients who took part in the study willingly with written consent were interviewed. Patient's confidentiality was strictly maintained. Patient's personal data regarding name, age, sex, and other information were not disclosed anywhere and were used for research purposes only.

Consent for publication

Not applicable.

Availability of data

The data that support the findings of this study are not publicly available due to the presence of information that could compromise the privacy of research participants but are available from the corresponding author.

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ACCSCIENCE PUBLISHING

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ORIGINAL RESEARCH ARTICLE

BDNF-AS as a promising downregulated biomarker for multiple sclerosis pathogenesis and diagnosis

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Abstract

Multiple sclerosis (MS) is a prevalent neurological disorder affecting the central nervous system, with a global incidence exceeding 2.5 million cases. Brain-derived neurotrophic factor-antisense (*BDNF-AS*), a long non-coding RNA, has been identified as a factor that negatively influences the expression of brain-derived neurotrophic factor, a neurotrophin protein that exhibits heightened expression within actively demyelinating lesions in MS. This study aims to assess the relative expression of *BDNF-AS* across all MS subtypes, evaluate its diagnostic accuracy, and explore correlations between *BDNF-AS* expression and disease parameters. Quantitative real-time polymerase chain reaction was employed to quantify the expression levels of *BDNF-AS* in the serum samples of 54 individuals diagnosed with various types of MS and 20 healthy controls. Statistical analyses were performed to assess the correlation and diagnostic efficacy of *BDNF-AS* expression levels. The expression of *BDNF-AS* was markedly reduced in individuals with MS compared to the control group ($P < 0.01$). Notably, the highest expression levels were observed in patients diagnosed with secondary progressive MS. Using a defined cutoff value of 0.31, and the findings suggest that *BDNF-AS* expression in serum has notable potential as a specific and sensitive diagnostic marker for MS. In conclusion, this study provides a comprehensive evaluation of *BDNF-AS* across all MS subtypes, highlighting its diagnostic accuracy and the association between elevated *BDNF-AS* expression and disease progression in secondary progressive MS. Further research is needed to validate these results.

Keywords: Multiple sclerosis; Brain-derived neurotrophic factor; Brain-derived neurotrophic factor-antisense; Relapsing-remitting multiple sclerosis; Secondary progressive multiple sclerosis; Primary progressive multiple sclerosis; Gene expression

1. Introduction

Neurodegeneration refers to the progressive loss of neuronal function within the central nervous system (CNS), affecting millions of people and posing a major medical challenge worldwide.[1] Multiple sclerosis (MS) is a neurodegenerative condition that results in the degeneration of neuronal axons and gliosis within the CNS.[2] According to the 2013 MS Atlas, the Middle Eastern, and North African nations are classified as low- to moderate-risk regions for MS.[3] It is characterized by ongoing inflammation within the CNS, which results in tissue damage, leading to brain and spinal cord atrophy and irreversible neurological impairment.[4] MS is considered the most prevalent neurological disease among young adults, affecting more than 2.5 million people globally.[5] In Africa and the Middle East, Egypt has the highest incidence of MS, with 59,671 documented patients.[6]

The majority (~85 – 90%) of MS cases initially present a relapsing-remitting disease course (i.e., RRMS), whereas 10% of cases develop primary progressive MS (PPMS). About 90% of RRMS patients eventually progress to secondary progressive disease (i.e., SPMS).[7] The causes of progressive MS are not as well understood as those of relapsing forms, which involve disruptions in the immune system.[8] While inflammation is the primary driver of MS activity, neurodegeneration is also thought to play a major role in the disease progression.[9]

Long non-coding RNAs (lncRNAs) are transcripts longer than 200 nucleotides that do not have the functional ability to code for proteins.[10,11] The functions of lncRNAs, such as those of protein-coding genes, are diverse and can vary greatly.[12] LncRNAs have been shown to play a role in various significant biological processes through interactions with DNA or chromatin, modulation of regulatory or signaling pathways, and the regulation of other types of ribonucleic acid (RNAs).[13] Some lncRNAs may function as decoys, binding to effector proteins and preventing them from carrying out their intended functions by diverting them from their target sites.[10] Certain lncRNAs contain so-called "microRNA (miRNA) response elements," which are complementary binding sites for miRNAs. These lncRNAs are known as competitive endogenous RNAs (ceRNAs), which act as molecular sponges for miRNAs, inhibiting their ability to reach target genes.[14] Despite typically having lower expression levels than messenger RNAs, lncRNAs often display more distinctive expression patterns specific to certain tissues and cells, suggesting that they play crucial roles in cell-specific pathways.[15]

The CNS is composed of a variety of cell types with distinct morphologies and functions. Neurotrophic lncRNAs are believed to influence the genetic and epigenetic regulation of the expression of genes involved in the CNS.[16,17] Numerous lncRNAs expressed in the CNS have been found to play critical roles in the functions of neural cells, neurodevelopment, and the regeneration and differentiation of neural stem cells.[18] Brain-derived neurotrophic factor-antisense (*BDNF-AS*), a type of lncRNA, is known for its role in regulating the expression and function of the neurotrophin protein brain-derived neurotrophic factor (BDNF) within the CNS.[19,20]

The downregulation of *BDNF-AS* has been shown to inversely upregulate BDNF protein levels, resulting in a pro-neuronal effect by stimulating neuronal cell growth and differentiation.[21,22] This effect is believed to be mediated by BDNF, which is recognized as a critical regulator of neuronal plasticity, making it one of the most significant proteins in this context.[23] Importantly, BDNF is overexpressed by immune cells in MS lesions,[24] and other studies have demonstrated that BDNF protein levels are reduced simultaneously in the serum or plasma of MS patients compared to controls.[25-30] Therefore, the level of *BDNF-AS* has become an important focus of research in MS in investigating its role in regulating BDNF in MS and its potential pathophysiological function.

To our knowledge, the expression of *BDNF-AS* has not been thoroughly investigated in neurological diseases within patient blood samples; it has only been analyzed in cell models and postmortem patient tissues. Moreover, the only study on *BDNF-AS* in MS focused on whole blood from RRMS patients, where no significant differences were found compared to controls.[31] Therefore, our study aims to be the first to comprehensively investigate the expression of *BDNF-AS* across different MS subtypes and explore its relationship with disease prognostic parameters. This research will contribute to understanding the role of BDNF in MS and examine the diagnostic and prognostic potential of *BDNF-AS* in MS.

2. Methods

2.1. Subject recruitment

This study involved 54 individuals diagnosed with MS at multiple stages of the disease alongside 20 healthy volunteers, carefully matched for gender and age. Recruitment occurred between January and May 2021, with participants sourced from the MS Research Unit at Cairo University Hospital in Kasr Al-Ainy, Cairo, Egypt. Written informed consent was obtained from all participants, both patients and controls, before their involvement. Ethical approval (approval number: 19922) was granted by the Board of Ethical Committee, Suez University, Egypt, ensuring compliance with the ethical principles outlined in the Declarations of Helsinki (1964) and its subsequent revisions.[32]

2.2. Characteristics of subjects

All participants were diagnosed with MS based on the guidelines set forth by the International Panel on the Diagnosis of MS, employing the McDonald's criteria (2010).[33] A total of 74 participants were included in the study, comprising 31 cases from the RRMS group, 13 from the SPMS group, 10 from the PPMS group, and 20 healthy volunteers as controls. The RRMS and SPMS groups were subdivided as follows: 16 with relapsing RRMS, 15 with remitting RRMS, eight with relapsing SPMS, and five with remitting SPMS. The degree of clinical disability was evaluated using the Kurtzke expanded disability status scale (EDSS).[34]

2.3. Exclusion criteria

Individuals were deemed ineligible for the research if they had any of the following conditions: cancer, liver disease, diabetes mellitus, autoimmune disorders, hypertension, cerebrovascular disease, pregnancy, infectious or inflammatory diseases, a history of alcohol abuse, or smoking, based on the specified eligibility criteria.

2.4. Demographics and disease characteristics in MS subjects

Clinical data collected from all participants in this study were as follows:

(i) Frequency of relapses in the previous 3 years
(ii) Age and sex
(iii) EDSS score
(iv) History of family MS or other autoimmune diseases
(v) Current disease status (relapse/remitting) at the time of sampling
(vi) History and duration of MS
(vii) Patient's clinical history.

2.5. Specimen collection

Five milliliters of blood were drawn from both MS patients and the 20 healthy volunteers. The blood samples were incubated for 30 min at 37°C and then centrifuged for 10 min at 3000 rpm. The resulting serum was divided into two fractions and stored at −80°C until required for analysis.

2.6. RNAs extraction

RNAs were extracted using the QIAGEN serum and plasma RNA extraction kit (catalog number 217184, Hilden, Germany). A 200 µL serum aliquot was processed according to the manufacturer's instructions. Approximately 14 µL of RNA was eluted, and the purity and concentration were assessed using the One/One^{C} Microvolume ultraviolet-Vis NanoDrop™ Spectrophotometer (ThermoFisher, Singapore). The eluted RNA was stored at −80°C until needed.

2.7. Complementary deoxyribonucleic acid (cDNA) synthesis

cDNA was synthesized from 2.5 µg of total RNA using the QIAGEN RT² First Strand Kit (catalog number 330404, Hilden, Germany), following the manufacturer's guidelines. The process involved three stages: (i) genomic DNA removal at 42°C for 5 min, (ii) reverse transcription at 37°C for 60 min to optimize reverse transcriptase activity, and (iii) enzyme denaturation at 95°C for 5 min. All thermal cycles were performed using the SimpliAmp™ Thermal Cycler (Applied Biosystems, Singapore).

2.8. Real-time polymerase chain reaction (PCR)

Real-time PCR was used to evaluate the relative gene expression of the target gene (*BDNF-AS*) and the housekeeping gene (*GAPDH*). Pre-designed primers from QIAGEN (catalog number 330701, Hilden, Germany) were utilized, with the following identifications for each gene: *GAPDH* (LPH15126A-200) and *BDNF-AS* (LPH15814A-200). The amplification was carried out using QIAGEN's RT² SYBR Green ROX quantitative PCR Mastermix (catalog number 330520, Hilden, Germany). Forty amplification cycles were performed, each consisting of 15 seconds at 95°C and 1 min at 60°C. After amplification, a melting curve analysis was performed to assess the PCR products. All processes were carried out using the Real-Time PCR QuantStudio™ 1 (Applied Biosystems, Singapore).

2.9. Relative gene expression

The relative expression of *BDNF-AS* was calculated for each sample using the double-delta cycle threshold (Ct) method. The Ct values of all samples were normalized against *GAPDH*. The fold change for each sample was calculated and compared between patients and normal controls to investigate the relative expression of *BDNF-AS*.

2.10. Statistical analysis

Statistical analyses were conducted using Statistical Package for the Social Sciences Statistics (IBM, version 26, USA). A normality test was performed on all data to differentiate between non-parametric and parametric data. The non-parametric Kruskal–Wallis test was utilized to evaluate variations among groups. Multiple comparison tests were applied to identify the source of any significant differences between groups. The non-parametric Spearman's correlation test was used for correlation analysis. Receiver operating characteristic (ROC) curve analysis was also performed.[35] A $P < 0.05$ was considered statistically significant. Microsoft software was used to create all graphs and tables.

3. Results

3.1. Demographics and disease characteristics in MS subjects

When comparing age and sex across the disease groups and the control group, no differences were observed, indicating that all groups were age- and sex-matched. Regarding family history of MS, only 2 cases (6.5%) within the RRMS group and 1 case (7.7%) within the SPMS group reported a family history of the disease. The relapse/remitting classification within the RRMS and SPMS groups revealed 16 relapse RRMS cases, eight relapse SPMS cases, 15 remitting RRMS cases, and five remitting SPMS cases. The number of relapses within the past 3 years (median ± interquartile range) was 2 ± 2 for RRMS and 4 ± 2 for SPMS. The mean ± standard deviation of the EDSS scores were 3.05 ± 2.74 for RRMS, 6.00 ± 1.02 for SPMS, and 5.55 ± 1.14 for PPMS. The demographic and clinical characteristics of all included subjects and controls are summarized in Table 1.

3.2. Evaluation of glyceraldehyde-3-phosphate dehydrogenase as a suitable reference gene

The suitability of *GAPDH* as a reference control gene was assessed by examining the average Ct across all MS patients and healthy control volunteers. The mean Ct values (± standard error [S.E.]) for *GAPDH* were as follows: 25.97 ± 0.058 for control samples, 23.69 ± 0.78 for PPMS, 24.59 ± 0.34 for RRMS-relapse, 24.58 ± 0.37 for RRMS-remitting, 25.03 ± 0.74 for SPMS-relapse, and 27.36 ± 0.49 for SPMS-remitting. The normality of the data was confirmed using the non-parametric Shapiro–Wilk and Kolmogorov–Smirnov tests, with $P = 0.153$ and 0.200, respectively. A parametric one-way analysis of variance was conducted to examine differences in the Ct values of *GAPDH* across all groups, revealing no statistically significant differences ($P = 0.068$). These findings are illustrated in Figure 1, which shows the expression of *GAPDH* in all MS patient groups and healthy controls.

3.3. Relative expression of *BDNF-AS*

The lncRNA *BDNF-AS* was found to be significantly downregulated ($P < 0.01$) in MS patients compared to control subjects. The fold change relative to the control (mean ± S.E) was 0.19 ± 0.06 for PPMS, 0.33 ± 0.07 for RRMS-relapse, 0.30 ± 0.08 for RRMS-remitting, 0.33 ± 0.11 for SPMS-relapse, and 0.83 ± 0.20 for SPMS-remitting. Multiple comparison tests revealed that all groups were significantly different from the control, except for the SPMS-remitting group, which showed no significant difference from the controls and exhibited the highest expression among all groups. However, there were no

Table 1. Disease characteristics and demographics of multiple sclerosis subjects and controls

Group	Age[a]	Age at onset[a]	Gender[c]		Family history[c]		Status[c]		MS duration[a]	Frequency of relapses[b,d]	EDSS[a]
			Male (%)	Female (%)	Yes (%)	No (%)	Relapse (%)	Remitting (%)			
RRMS (n=31)	31.10±9.04	25.68±7.54	13 (42)	18 (58)	2 (6.5)	29 (93.6)	16 (51.6)	15 (48.4)	5.11±4.65	2±2	3.05±2.74
SPMS (n=13)	46.38±9.49	31.23±7.67	6 (46)	7 (54)	1 (7.7)	12 (92.3)	8 (61.5)	5 (38.5)	15.12±8.66	4±2	6.00±1.02
PPMS (n=10)	45.10±10.49	34.30±4.16	4 (40)	6 (60)	0 (0)	10 (100)	-	-	10.8±9.9	-	5.55±1.14
Control (n=20)	37.70±16.84	-	9 (45)	11 (55)	0 (0)	20 (100)	-	-	-	-	-
P-value	0.382	-	0.883		-	-	-	-	-	-	-

Notes: [a]Mean±standard deviation; [b]Median±interquartile range; [c]Frequency; [d]Within the last 3 years. Abbreviations: EDSS: Expanded disability status scale; MS: Multiple sclerosis; PPMS: Primary progressive multiple sclerosis; RRMS: Relapsing-remitting multiple sclerosis; SPMS: Secondary progressive multiple sclerosis.

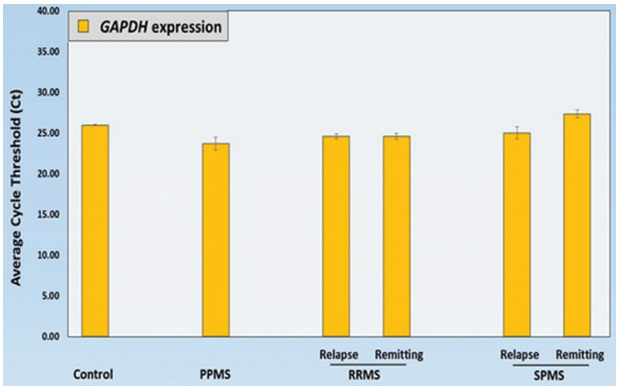

Figure 1. Average cycle threshold (Ct) values of *GAPDH* in multiple sclerosis patients and healthy controls. The normality of the data was assessed using the non-parametric Shapiro–Wilk and Kolmogorov–Smirnov tests, yielding non-significant $P = 0.153$ and 0.200, respectively. A parametric one-way analysis of the variance test showed no statistically significant differences in the Ct values of *GAPDH* across all groups ($P = 0.068$). Error bars represent standard errors.
Abbreviations: PPMS: Primary progressive multiple sclerosis; RRMS: Relapsing-remitting multiple sclerosis; SPMS: Secondary progressive multiple sclerosis.

significant differences between the MS groups themselves. These results are depicted in Figure 2.

3.4. ROC curve analysis

Based on the above findings, a ROC curve was generated, and the area under the curve (AUC) was calculated to evaluate the diagnostic value of *BDNF-AS*. The calculated AUC (mean ± S.E.) was 0.869 ± 0.041, with a 95% confidence interval of 0.789 – 0.948. The optimal cutoff point, determined by the highest Youden index value across all possible cutoffs, was 0.31, which corresponds to 75.93% sensitivity and 100% specificity. These findings are summarized in Figure 3.

3.5. Correlation analysis

Spearman's non-parametric correlation test was used to examine the relationship between the relative expression of the *BDNF-AS* gene and various factors, including EDSS, age, MS duration, age at onset, and frequency of relapses in the previous 3 years. The analysis revealed a significant, moderately positive correlation with age at onset of MS ($r = 0.384$, $P < 0.01$), but no correlation was found with any of the other factors. These findings, along with the correlation parameters, are presented using the four-parametric logistic regression model, as shown in Figure 4.

4. Discussion

The investigation into the expression of *BDNF-AS* was prompted by its established role in the negative regulation of the neurotrophic protein BDNF, which exhibits complex and intriguing behavior in the context of MS and other neurodegenerative diseases.[36] First, it was found that immune cells secrete BDNF in MS lesions but only in those with active myelin degeneration.[24] Second, despite this finding, studies have shown that BDNF levels in the serum/plasma, cerebrospinal fluid (CSF), and peripheral blood mononuclear cells are lower in MS patients.[25-30] Interestingly, BDNF levels in the SPMS group were found to be lower than in the RRMS and the PPMS groups.[27] A similar pattern of low BDNF levels in the serum has been observed in other neurological diseases, such as schizophrenia,[37,38] depression,[39,40] Alzheimer's disease,[41,42] and Huntington's disease.[43,44] In contrast, autism has been associated with higher serum levels of BDNF.[45]

A study by Modarresi *et al.*[46] demonstrated that inhibiting *BDNF-AS* can increase endogenous BDNF protein expression, stimulate neurite formation, and promote neuronal differentiation. Therefore, in our study, we aim to comprehensively examine the levels of *BDNF-AS* in the serum of MS patients across all subtypes of the disease. Interestingly, we found that *BDNF-AS* levels were significantly lower in the serum of MS patients compared to healthy controls. This observation may reflect a downregulation mechanism that inhibits *BDNF-AS* to upregulate BDNF protein expression in MS lesions, potentially stimulating synaptic plasticity, and myelin regeneration in MS plaques as part of an attempt to recover from the demyelinating event characteristic in MS.

One interesting finding that might contradict this explanation is the downregulation of BDNF itself in the serum of MS patients.[25-29] This observation could challenge our hypothesis that the downregulation of *BDNF-AS* facilitates overexpression of BDNF in MS. However, the reduced levels of BDNF in the serum or CSF of MS patients.[28,29] may suggest that BDNF is predominantly localized within MS lesions rather than being secreted into body fluids. Therefore, the overexpression of BDNF resulting from *BDNF-AS* downregulation may occur only in MS lesions, where this mechanism is activated. Alternatively, this observation may indicate the presence of another compensatory mechanism that mimics the effects of *BDNF-AS* in inhibiting BDNF. Nevertheless, our initial hypothesis remains the most plausible explanation, as any compensatory mechanism would likely influence BDNF levels in MS lesions, which is not observed given the higher expression of BDNF in these lesions.

Our finding of lower expression of the *BDNF-AS* in MS, whereas BDNF remains downregulated, is in line with a study by Gharzi *et al.*[31] on RRMS patients, which found that the correlation between BDNF and *BDNF-AS* may not always be negative. This finding may extend to

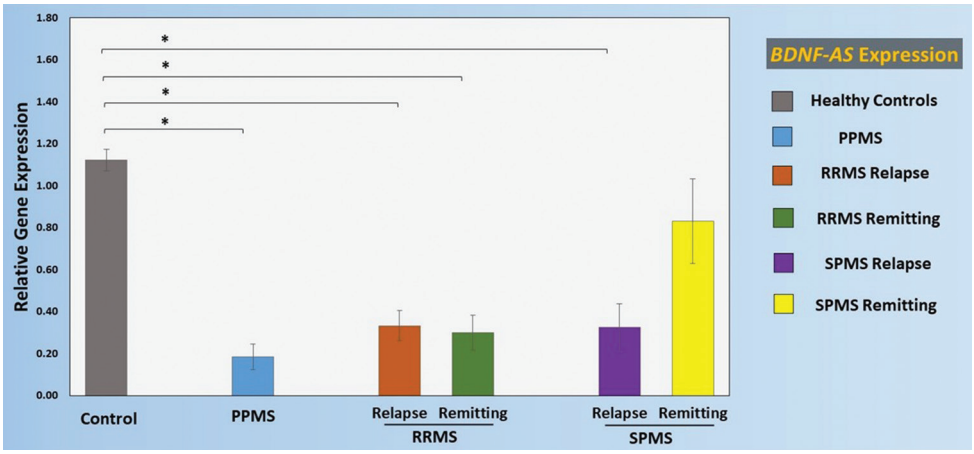

Figure 2. Relative expression of brain-derived neurotrophic factor-antisense multiple sclerosis subjects compared to healthy controls. Data are presented as mean and standard error. The figure shows that PPMS, RRMS-relapse, RRMS-remitting, and SPMS-relapse are statistically downregulated compared to the control group. However, SPMS-remitting is not statistically different from the controls. Additionally, no significant differences were observed among the multiple sclerosis types themselves.
Abbreviations: PPMS: Primary progressive multiple sclerosis; RRMS: Relapsing-remitting multiple sclerosis; SPMS: Secondary progressive multiple sclerosis.

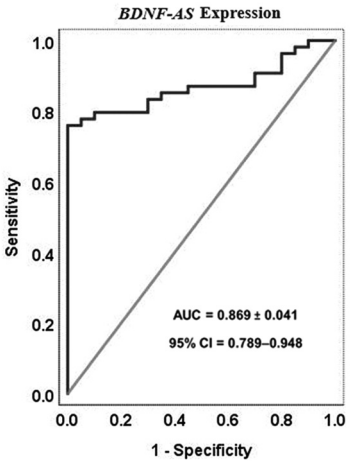

Figure 3. Diagnostic performance of brain-derived neurotrophic factor-antisense using receiver operating characteristic curve analysis. The optimal cutoff was calculated to be 0.31, with a sensitivity of 75.93%, specificity of 100%, and an overall AUC of 0.869 ± 0.041.
Abbreviations: AUC: Area under the curve; CI: Confidence interval.

other diseases, such as bipolar disorder, gastric cancer, and breast cancer, where studies have suggested that a positive correlation between BDNF and *BDNF-AS* may also exist despite the known negative regulation of BDNF by *BDNF-AS*.[19,31]

Previous studies have demonstrated that BDNF is highly produced in RRMS, whereas its expression is lower in progressive MS.[24,26] This finding could be explained by hypothesizing that in progressive MS, BDNF is more negatively regulated by *BDNF-AS*. One potential consequence of this regulation is the progression of neuronal degeneration in progressive types of MS. Our study supports this hypothesis, as the SPMS group exhibited the highest *BDNF-AS* expression among MS subtypes and was the only group not showing significant downregulation compared to controls. On the other hand, the PPMS group demonstrated low *BDNF-AS* expression, suggesting that the progression of the disease in this group may involve a different mechanism than the one hypothesized for the SPMS group.

Moreover, we found that *BDNF-AS* expression displayed higher specificity and sensitivity for MS diagnosis, which may prove useful in diagnosing MS. We also determined a relative expression cutoff of 0.31, which demonstrated moderate sensitivity and high specificity. However, our study failed to find significant differences between the RRMS, PPMS, and SPMS groups, which may be necessary for distinguishing between different MS types.

5. Limitations and future research

It is important to acknowledge that every study has its limitations, which often present valuable opportunities for future research and deeper exploration. First, the sample size for each group was constrained by rigorous exclusion criteria (Section 2.3), designed to ensure high specificity by excluding cases with confounding medical conditions that could influence lncRNA expression. Moreover, although the samples were drawn from the MS Unit at Kasr Al-Ainy in Cairo – a center that attracts patients from across Egypt due to the scarcity of specialized MS facilities – the geographic scope of the study was inherently limited to a single country. While this regional focus provides a representative view of MS patients within Egypt, it highlights the need for broader, multicenter studies

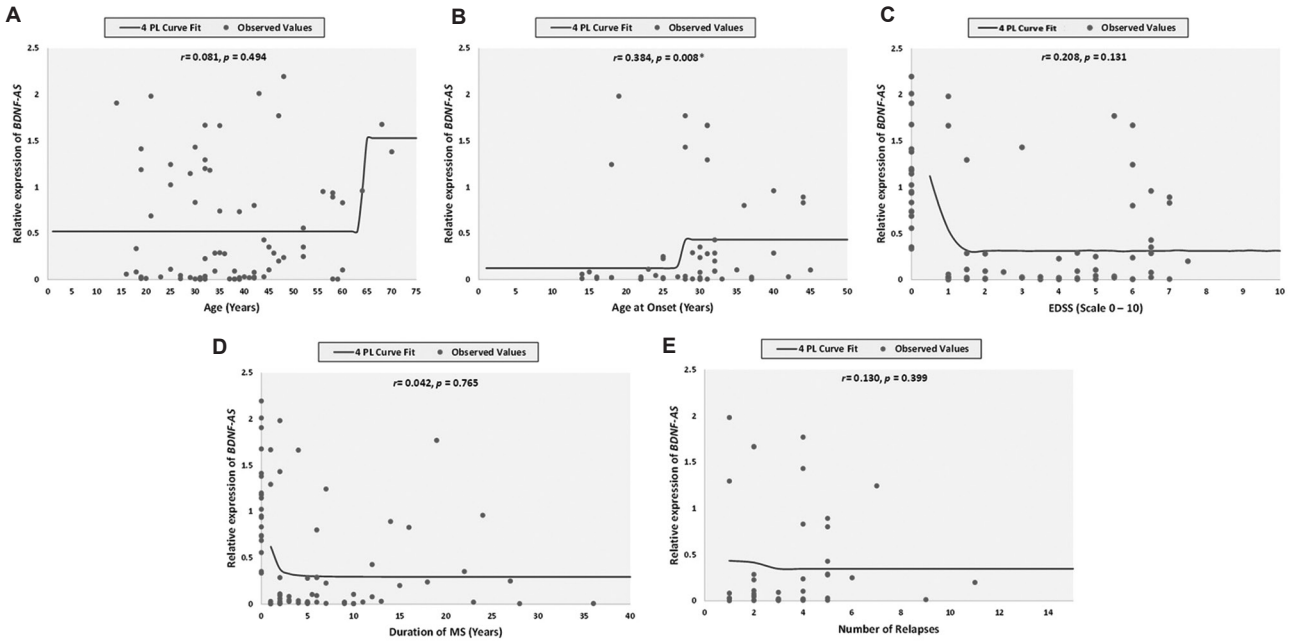

Figure 4. Correlation of brain-derived neurotrophic factor-antisense (*BDNF-AS*) expression with (A) age, (B) age at onset, (C) Expanded Disability Status Scale (EDSS), (D) multiple sclerosis (MS) duration, and (E) incidence of relapses in the preceding 3 years. The four-parametric logistic (4PL) model was used to fit the data, and Spearman's test was applied to investigate the non-parametric correlation parameters. *BDNF-AS* expression had a moderately positive and significant correlation with age at onset of MS ($r = 0.384$, $P < 0.01$).
Notes: *P*: *P*-value; r: Correlation coefficient.

to validate the generalizability of these findings across diverse populations. In addition, the variation in treatment regimens among patients with different MS subtypes raises intriguing questions about the potential impact of therapeutic approaches on *BDNF-AS* expression. This question highlights the importance of future investigations to examine the interplay between treatment modalities and lncRNA expression.

While this study highlights the downregulation of *BDNF-AS* across various MS subtypes, the precise mechanisms underlying this phenomenon remain incompletely understood. Of particular interest is the marked elevation of *BDNF-AS* expression in SPMS compared to other groups, particularly PPMS, which raises important questions regarding its role in disease progression. Although limited funding constrained our ability to explore this aspect further, future studies investigating these differences could yield critical insights into the molecular pathways and key regulatory mechanisms driving MS pathophysiology and progression. Finally, measuring BDNF protein levels alongside *BDNF-AS* expression would offer a more comprehensive understanding of the regulatory network. Although financial constraints precluded this aspect of the study, we have addressed this limitation by integrating data from existing literature to contextualize our findings (Section 4). Expanding such investigations in future studies could significantly enhance our understanding of the interplay between BDNF and *BDNF-AS* in MS pathophysiology and progression.

While these considerations represent opportunities for further exploration rather than limitations, they underscore the need for continued research to build upon the foundation established by our study.

6. Conclusion

The expression of *BDNF-AS* has been identified as a potential biomarker for the diagnosis of MS, demonstrating high specificity and sensitivity through its downregulation in the serum of MS patients. Furthermore, our findings suggest that elevated levels of *BDNF-AS* are associated with disease progression in secondary progressive MS, indicating that targeting the downregulation of this lncRNA could serve as a potential therapeutic strategy for MS. While these results are promising, further studies with larger sample sizes and across various MS subtypes are necessary. Future research should also investigate the expression of *BDNF-AS* in cell cultures, as well as brain and spinal cord samples from MS patients, to better understand its relationship with BDNF protein in CNS lesions and to gain deeper insights into disease mechanisms and potential treatment options.

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Conflict of interest

The authors declare that they have no competing interests.

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Methodology: Ahmed Kamal, Menha Swellam, Eslam M. El-Nahrery
Writing-original draft: Ahmed Kamal
Writing-review & editing: Menha Swellam, Eslam M. El-Nahrery

Ethical approval and consent to participate

The authors declare that this research adhered to the ethical principles outlined in the Declarations of Helsinki (1964) and its subsequent amendments [21]. Ethical approval (approval number: 19922) was granted by the Board of Ethical Committee, Suez University, Egypt. In addition, written informed consent was obtained from all patients and healthy controls after the nature of the study was fully explained.

Consent for publication

All patients were assured of data protection and privacy, except for their analytical data and clinical status, which were included in this study with their informed consent for use and publication.

Availability of data

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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INNOSC Theranostics and Pharmacological Sciences

CASE REPORT

Use of dexamethasone in septic cavernous sinus thrombosis coexisting with bacterial meningitis secondary to sinusitis: A case report

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Abstract

We present the case of a 69-year-old woman who developed septic cavernous sinus thrombosis (SCST) secondary to bacterial meningitis and sinusitis. She presented to the emergency department with frontal headache, neck stiffness, photophobia, exophthalmos, and fever. Initial investigations revealed extensive opacification of the paranasal sinuses on plain cranial computed tomography (CT), and blood tests indicated a bacterial infection. She initially received antibiotics and dexamethasone for suspected bacterial meningitis. Cerebrospinal fluid analysis revealed elevated glucose and protein levels as well as white and red blood cell counts, along with a negative Gram stain. An ophthalmology assessment and CT venography indicated a strong suspicion of cavernous sinus thrombosis, further confirmed by magnetic resonance venography. Anticoagulation with antibiotics and dexamethasone was initiated, leading to significant clinical improvement. This case highlights the importance of the early recognition and treatment of SCST and the role of dexamethasone in patients with coexisting bacterial meningitis.

Keywords: Septic cavernous sinus thrombosis; Dexamethasone; Corticosteroids; Bacterial meningitis; Sinusitis; Antibiotics; Anticoagulation

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1. Introduction

Cavernous sinus thrombosis (CST) is a rare yet serious condition that may complicate infections such as sinusitis, orbital cellulitis, and pharyngitis, particularly after a thrombophilic event. It can present as septic or aseptic. The primary etiological organisms are bacteria, with *Staphylococcus aureus* accounting for approximately two-thirds of cases. Other frequently encountered pathogens include *Streptococcus* species.[1]

CST commonly arises from the hematogenous spread of infection to the cavernous sinus through the venous system, particularly via sphenoid sinusitis. The pterygoid plexus, a valveless venous system that connects with the cavernous sinus, increases the susceptibility to impaired drainage. The fine, irregular structure of the cavernous sinus functions as a sieve, trapping bacteria and increasing the risk of thrombophlebitis.[2]

2. Case presentation

A 69-year-old Caucasian woman presented to the emergency department after a referral from her primary care physician, with a 3-day history of progressively severe

frontal headache, facial pain, fever, neck rigidity, blurred vision, and photophobia. Her medical history included chronic obstructive pulmonary disease, hypertension, and hyperlipidemia. She had no recent travel history or infection contact.

On examination, she was alert but appeared drowsy. She had significant exophthalmos in the right eye. Both pupils were reactive to light, and eye movements were normal. She also had mild chemosis. Cranial nerves were grossly intact. She had neck stiffness and a positive Brudzinski sign. Her muscle power and reflexes were normal. On auscultation, she had mild wheezing and normal cardiovascular parameters and had no rashes.

Initial laboratory workup demonstrated a high level of C-reactive protein (366 mg/L [0 – 10 mg/L]) and white blood cell (white blood cells [WBC]; 32.1 × 10^9/L [4 – 11 × 10^9/L]) and neutrophil [30.8 × 10^9/L (2 – 7.5 × 10^9/L)] counts. Cranial computed tomography (CT) revealed extensive opacification of the paranasal sinuses (Figure 1). Given her clinical presentation and laboratory and imaging findings, the leading differential diagnosis was sinusitis with a high suspicion of meningeal involvement, likely bacterial in origin. She was empirically treated with antibiotics intravenously, particularly chloramphenicol and cotrimoxazole (she was allergic to penicillin). Intravenous dexamethasone, 10 mg every 6 h for 4 days, was also initiated.

On day 2, an ophthalmology consultation was requested due to persistent exophthalmos, chemosis, and reduced visual acuity. Subsequent CT venography (CTV) revealed a small filling defect in the right internal jugular vein (Figure 2) and right cavernous sinus (Figure 3), which was suggestive of a thrombus and raised a strong suspicion of CST.

Moreover, contrast-enhanced magnetic resonance venography (MRV) revealed a filling defect in the right cavernous sinus, confirming the diagnosis of right CST (Figure 4).

Cerebrospinal fluid (CSF) analysis revealed high levels of glucose (4.4 mmol/L) and protein (1 g/L), as well as WBC (18 cells/μL) and red blood cell (26 cells/μL) counts, along with xanthochromia and a negative Gram stain. CTV and MRV were further reviewed by an interventional neuroradiologist, who agreed with the diagnosis of meningitis and CST without evidence of subarachnoid hemorrhage. A remote neurosurgical consultation was sought, and therapeutic full-dose low-molecular-weight heparin (LMWH) was recommended. Blood culture identified *Streptococcus intermedius* sensitive to penicillin; however, due to the patient's penicillin allergy, targeted

Figure 1. Cranial computed tomography highlighting extensive opacification of the paranasal sinuses – marked opacification of the right sphenoid (blue arrow), left sphenoid, and ethmoid sinuses consistent with sinusitis

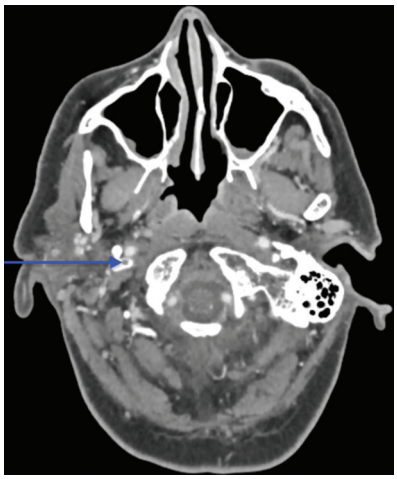

Figure 2. Computed tomography venography. The blue arrow highlights a small filling defect in the right internal jugular vein that appears darker (hypodense) than the left internal jugular vein, which appears well-opacified and bright, indicating normal blood flow with contrast filling. This darker appearance on the right indicates the absence of contrast filling in the vein, suggesting thrombus.

antibiotic therapy with chloramphenicol and cotrimoxazole was continued in accordance with institutional protocols and microbiologist advice.

The patient's presentation, laboratory data, and imaging findings were consistent with bacterial meningitis with secondary CST (SCST). Treatment decisions were made following established protocols for bacterial meningitis and SCST, which included commencing antibiotics, anticoagulation, and dexamethasone.

By hospital day 4, the patient had shown significant clinical improvement. Her neck pain and headaches

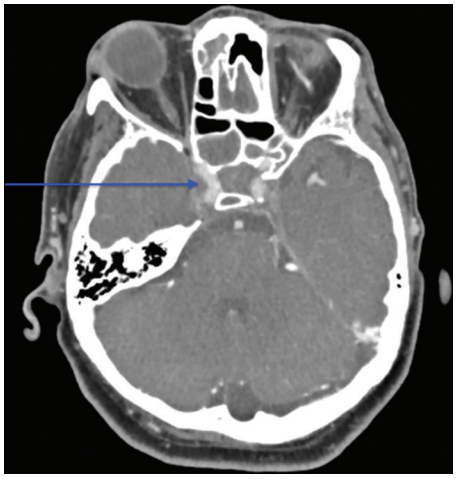

Figure 3. Computed tomography venography showing a cross-sectional view of the head, with a focus on the cavernous sinus on either side of the sella turcica. The blue arrow points to the right cavernous sinus, which appears darker and less enhanced. This hypoattenuation indicates a filling defect in the right cavernous sinus, which is suggestive of thrombosis in contrast to the left cavernous sinus that shows bright enhancement consistent with normal contrast uptake and unobstructed blood flow.

Figure 4. Magnetic resonance venography showing hypointensity on the right cavernous sinus (blue arrow) contrasting with the left side (yellow arrow) that remains bright. Normally, both cavernous sinuses appear bright due to blood flow. This filling defect on the right indicates stagnant or absent flow within the right cavernous sinus, which is consistent with cavernous sinus thrombosis

subsided. She remained on intravenous antibiotic and anticoagulation therapies for 2 weeks while in the hospital and was discharged on oral antibiotics for another 2 weeks and apixaban, with a plan to review anticoagulation on follow-up.

At her follow-up review after discharge, she reported no new or re-emergent symptoms. However, she had persistent mild headaches, which were not as severe as her initial presentation; accordingly, she was started on a trial of amitriptyline and planned for repeat MRV in 6 months to assess resolution and remained on anticoagulation therapy.

3. Discussion

The prevalence of SCST has markedly declined in the antibiotic era, and its mortality rate has significantly reduced to <30% in the postantibiotic era. However, it remains potentially lethal with long-term complications.[3] The subtle nature of early symptoms frequently results in delayed recognition and treatment initiation, causing guarded prognostication in SCST.[4] Magnetic resource imaging (MRI) and MRV are the preferred imaging modalities for diagnosing cerebral venous thrombosis (CVT).[3] Identifying CST complicating bacterial meningitis is particularly challenging due to overlapping clinical presentations.[5]

Because anticoagulation helps prevent thrombus expansion, has anti-inflammatory properties, and promotes the penetration of antibiotics into the thrombus, it has been proposed to treat SCST. Anticoagulation in SCST is beneficial in reducing mortality, improving neurological outcomes, and decreasing residual morbidity, particularly with early initiation of treatment.[6-8] However, anticoagulation is associated with risks of intracranial and systemic hemorrhage. A meta-analysis of patients with CVT indicated a 13% reduction in the mortality rate or dependency without an increase in hemorrhagic events, even among those with pre-existing intracranial hemorrhage, supporting the cautious use of anticoagulation in CST.[9] A recent systematic review and meta-analysis of 72 studies analyzing individual data of 110 patients (of which 60 had anticoagulation, with heparin being the most commonly used, followed by LMWH and warfarin) on anticoagulation for CST revealed an adjusted odds ratio for mortality of 0.067 ($p = 0.007$) in the anticoagulant-given group. However, hemorrhagic complications occurred in two patients with hyperglycemia, indicating increased bleeding risk in this group and highlighting the need for caution in such cases. The large reduction in the adjusted odds ratio indicated that anticoagulation independently and significantly reduced the mortality risk in SCST despite the potential risk of hemorrhage.[10]

The European Federation of Neurological Societies recommends anticoagulation for 3 months for secondary cerebral venous and sinus thrombosis (CVST) with a transient risk factor, 6 – 12 months for idiopathic CVST and mild thrombophilia, and indefinitely for recurrent CVST or one CVST episode and severe thrombophilia.[11]

The use of corticosteroids in treating SCST is debated due to the inconclusive evidence of their efficacy,

prothrombotic properties, and tendency to exacerbate infection by suppressing the immune response. The benefits of decreasing orbital inflammation, cranial nerve edema, vasogenic edema, and intracerebral hemorrhage must be weighed against the adverse effects of corticosteroids. Despite limited literature supporting improved outcomes following corticosteroid administration, some studies have reported improved cranial nerve function resulting from decreased inflammation.[4,12] Notably, Ivey and Smith (1968) reported the usefulness of corticosteroids in treating adrenal insufficiency caused by pituitary dysfunction resulting from infarction or infectious spread from the cavernous sinus to sella turcica in the CST setting.[13] In 1962, Solomon *et al.* reported a case of CST that was diagnosed on hospital day 9. The condition of the patient progressed from unilateral, bilateral CST, with absolute ophthalmoplegia and bilateral ocular orbital inflammation over 6 weeks despite aggressive antibiotic and anticoagulation therapies. The patient subsequently showed improvement 2 days after the commencement of oral corticosteroids.[14] In this case, the infection might have been eliminated before starting corticosteroids, as they were initiated on day 37 when the clinical signs of sepsis had already subsided.

In 2008, Canhão *et al.* reported on the use of corticosteroids in CVT. Of the 624 adult patients included in the International Study on Cerebral Veins and Dural Sinus Thrombosis, 24% received corticosteroids with a median duration treatment of 11 days. They did not find evidence supporting the routine use of corticosteroids in the acute phase of CVT, except if indicated for treating an underlying disease other than CVT requiring corticosteroids. Corticosteroids were reported as possibly harmful and should be avoided in patients with CVT without CT or MRI evidence of parenchymal lesions.[15] Conversely, in 2014, Dinaker *et al.* reported a case of SCST with meningitis caused by community-acquired methicillin-resistant *S. aureus*, where the patient received antibiotics, anticoagulants, and dexamethasone, leading to rapid response and symptom resolution. At discharge, the patient had mild residual right hemiparesis and demonstrated full recovery at the 3-month follow-up.[16]

Given the rarity of SCST and limited literature regarding corticosteroids, we extrapolated data from other CVT studies and articles to help expand this topic. In our case, the patient showed significant improvement with corticosteroids and had no neurological deficit. Dexamethasone likely contributed to inflammation reduction, enhancing overall recovery. This supports the potential benefit of corticosteroids in managing CST with concurrent bacterial meningitis, where inflammation plays a critical role in symptomatology. However, further larger studies, particularly randomized controlled trials, may help clarify its benefits.

4. Conclusion

This case highlights the importance of early identification and aggressive management of SCST. The patient's substantial improvement may have been facilitated by the use of dexamethasone alongside antibiotics and anticoagulation. Despite the controversial role of corticosteroids in SCST, their administration in severe inflammation contributed to a positive outcome. This case supports the consideration of corticosteroids in similar clinical scenarios while emphasizing the need for further research into their role alongside conventional treatments in complex conditions such as SCST coexisting with meningitis. It also highlights the importance of a multidisciplinary approach in its diagnosis and management.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Verbal consent was obtained from the patient before her participation.

Consent for publication

Verbal consent was obtained from the patient to publish her data and scan images.

Availability of data

Not applicable.

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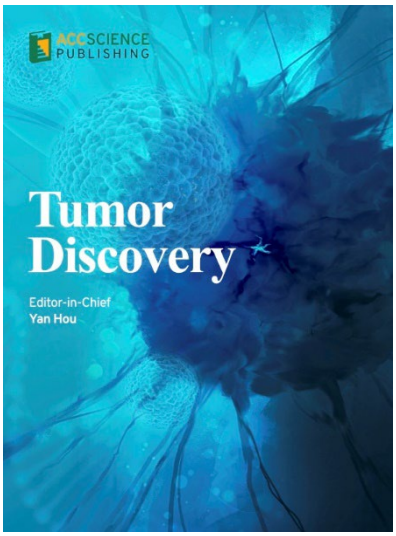

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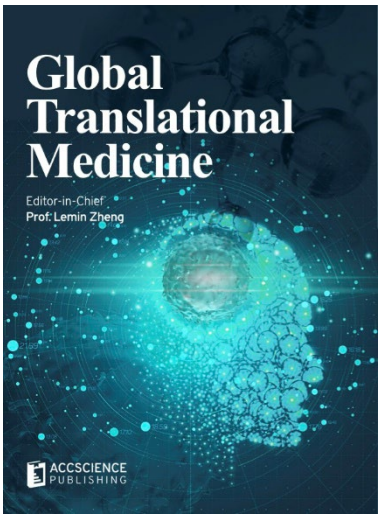

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