Beyond the Headlines: Why Henipaviruses Warrant Our Attention

Biplab Adhikari[1,*]

ABSTRACT

Henipaviruses, including Hendra and Nipah viruses, represent significant zoonotic threats with higher mortality rates. Due to limited therapeutic interventions, poses substantial challenges. These bat-borne pathogens were first identified in Australia (Hendra, 1994) and Malaysia (Nipah, 1998–1999), with subsequent multiple outbreaks. The recent discovery of Camp Hill virus in North American shrews, suggest broader geographic distribution than previously recognized.

Transmission occurs primarily through contact with reservoir hosts, though human-to-human spread has been documented in Nipah outbreaks. Initial non-specific febrile symptoms can progress to fatal encephalitis with distinctive pathological findings including syncytia formation and vasculitis. A concerning feature is the potential for relapsing encephalitis months or years after initial infection.

Management remains predominantly supportive, highlighting the urgent need for effective antivirals, vaccines, and enhanced surveillance. Expanded research into therapeutic countermeasures is essential to address this emerging global public health threat.

KEYWORDS

henipavirus; camp hill virus; Pteropus; hendra virus; nipah virus; encephalitis; flying fox; outbreak; shrew

AUTHOR AFFILIATION

[1] Department of Infectious Disease, University of Louisville School of Medicine, Louisville, Kentucky, USA

* Corresponding author: 600 Marshall Street, Louisville, KY, USA; biplabadhikari27@gmail.com

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INTRODUCTION

Highly pathogenic henipaviruses, which are characterized by their zoonotic origin, has been an evolving threat to human health and raise concern due to it limited therapeutic options. Hendra virus (HeV) and Nipah virus (NiV), members of genus henipaviruses, has high mortality rates around 60% and 92% respectively in humans (1). These HeV and NiV are bat-borne viruses whose clinical presentation may range from mild influenza-like symptoms to complications such as severe encephalitis and/or respiratory failure (2).

HISTORY

In 1994, the first reported case of henipavirus was reported in Australia when HeV caused a severe respiratory disease eruption in horses, concomitantly resulting in a few human fatalities (2). Soon after, in 1998–1999, an epizootic outbreak emerged following the identification of NiV in 283 human cases in Malaysia, which caused severe encephalitis with high mortality in 109 humans with and notable respiratory problems in pigs which served as amplifying intermediate host (2, 3). Since these initial outbreaks, there has been the progressive emergence of henipaviruses across wider geographic areas, particularly in the Asia-Pacific and African continents with significant public health consequences.

Smaller cluster outbreaks, although with higher mortality rates of NiV have primarily occurred in Bangladesh and India since 2001. Several other novel henipaviruses have been subsequently identified, including Cedar virus (CedPV), which is also a bat-borne virus that is non-pathogenic to animals and has been studied to be non-zoonotic (1, 2). Mojiang virus (MojV) was initially detected in a Rhinolophus cave-dwelling rats in China following three miners' death in 2012 from a severe pneumonia with acute respiratory distress syndrome. Furthermore, during the period from 2018–2022, multiple febrile patients in China were documented to have a Langya virus (LayV), a phylogenetically distinct shrewborne henipavirus that demonstrates significant zoonotic potential (4).

In January 2025, research team from the University of Queensland and Auburn University reported the first detection of a henipavirus, specified as Camp Hill virus (CHV) in North America. This virus was identified through the analysis of tissue samples obtained from four dead northern short-tailed shrews in 2021 from Tallapoosa County, Alabama (5). While no human infections with CHV have been documented thus far in epidemiological investigations, phylogenetic analysis revealed that CHV is genetically related to LayV which has previously demonstrated capacity for cross-species transmission from shrews to humans in China (5).

GENOMIC

Henipaviruses belongs to the Paramyxoviridae family (1). On a molecular level, henipaviruses exhibit a complex molecular structure and replication process that contribute to its significant pathogenicity. The viral genome is a single-stranded, negative-sense RNA approximately 18.2 kb in length which encodes six essential structural proteins (1). This genomic architecture supports the complexity of the viral life cycle and host interaction mechanisms. Henipavirus entry relies on a dual-protein system, in which the glycoprotein (G) binds to ephrin B2/3 receptors on host cells. These receptors are highly maintained across mammals and widely expressed in vascular endothelium and neurons (6, 7). Upon G protein binding, conformational changes activate the fusion (F) glycoprotein, which exists in a metastable pre-fusion state following proteolytic cleavage during virion maturation (8). In its activated form, the F protein undergoes structural reorganizations, exposing its hydrophobic fusion peptide. The insertion of this peptide into the host cell membrane initiates the formation of a fusion pore via which the viral ribonucleocapsid enters the host cell cytoplasm.

The virion's core comprises a ribonucleocapsid complex, in which nucleocapsid (N) proteins encapsulate the viral RNA genome that helps in protection and serve as a template for viral RNA synthesis (1). The ribonucleocapsid associates with the phosphoprotein (P) and large polymerase protein (L) to constitute the functional replication complex. During the viral budding process, a matrix (M) protein layer surrounding the viral ribonucleocapsid facilitates the interaction between the viral core and the host-derived envelope (7).

The viral P gene also encodes three non-structural proteins (C, V, and W) that are expressed in infected cells. These accessory proteins play a role in immune evasion mechanism, with the V protein specifically binding signal transducers and activators of transcription (STAT) molecules to prevent their nuclear translocation without degradation (7, 8). The W and C proteins contribute to immune evasion through mechanisms that are not yet fully understood.

TRANSMISSION

The spread of henipavirus between species occurs through multiple routes within the ecological setting. Fruit bats of the genus Pteropus (flying foxes) are known as the primary natural reservoir hosts for this virus, typically harboring viruses without exhibiting signs of disease (9). Although the infectivity of henipavirus is lower compared to that of the recent coronavirus (COVID-19) pandemic, its mortality rate is much higher-exceeding 60% for henipavirus, compared to mortality rate of less than 1% for COVID-19 globally (1, 10).

Several mechanisms have been explained for henipavirus transmission among species (Fig. 1).

SPILLOVER TRANSMISSION

There are multiple pathways that facilitate this spillover of henipavirus from the reservoir hosts. A Pteropid bat has been documented in most cases with direct and indirect spillover events (9). Bat behaviors such as roosting and

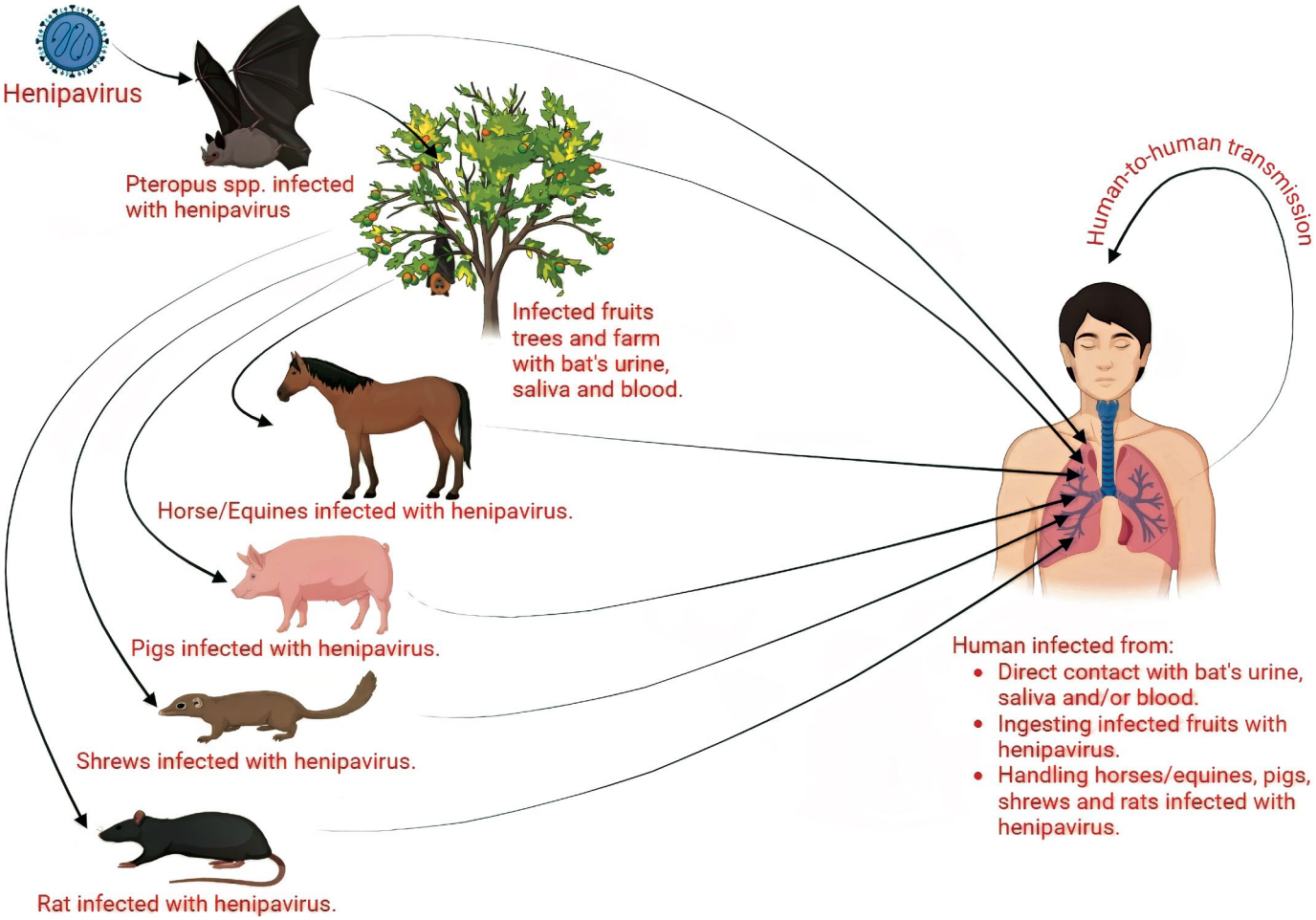

Fig. 1 Illustration of henipavirus transmission from its natural host, fruit bats (*Pteropus* spp.), to susceptible species. The arrows represent virus transmission within the natural reservoir and show up spillover events leading to disease.

feeding patterns create opportunities for virus shedding into the surrounding environment through saliva, urine, and/or excreta. These infectious materials may contaminate food sources consumed by intermediate hosts or humans, as it was reported during the transmission of NiV through palm sap consumption in Bangladesh (11).

Among domesticated animals, particularly pigs and horses have played significant roles as amplifying intermediate hosts in historical henipavirus outbreaks (1). In the case of NiV in Malaysia, intensive pig farming practices led to rapid viral transmission among swine populations, subsequently leading to human infections among farm workers and others in contact with infected animals (1). Likewise, HeV outbreaks in Australia was associated with horse infections followed by human transmission those in close contact with ill equines (1). These intermediate hosts often exhibit enhanced virus shedding and may facilitate viral adaptation through selective pressures, potentially increasing transmissibility to humans.

The molecular basis for interspecies transmission capability lays partly in the conservation of ephrin B2/B3 receptors across mammalian species (8). Additional factors such as viral genetic adaptations, host immune status, and ecological conditions collectively determine the success of cross-species infection. Relatively few mutations are reported to be required to enhance henipavirus transmission in new host species, emphasizing the evolutionary plasticity of these viruses and their potential for adaptation to different cellular environments (8, 12).

HUMAN-TO-HUMAN TRANSMISSION

Secondary route of transmission thru human-to-human has been documented in several NiV outbreaks in Bangladesh and India (11). This characteristic for sustained human transmission represents a concerning aspect of henipavirus epidemiology, as it eliminates the requirement for continued animal exposure once the virus has entered human communities. Close contact with infected individuals, particularly through caregiving activities, presents the highest risk for secondary transmission (1, 11).

Viral shedding through respiratory secretions, saliva, and other bodily fluids has been reported for human-to-human transmission. Patients with respiratory involvement may generate infectious aerosols during coughing or sneezing, facilitating airborne transmission in close-contact settings. Additionally, direct contact with infectious bodily fluids from patients with encephalitis or other symptoms can lead to transmission through mucous membrane exposure or percutaneous inoculation (1). Healthcare settings have emerged as significant amplification points for human-to-human spread, with nosocomial transmission documented in multiple outbreaks. Particularly when managing patients with suspected henipavirus

infections, it is important to follow proper infection control practices (11).

The reproductive number (R0) for human-to-human transmission of NiV has been estimated at approximately 0.5 in community settings, suggesting that sustained chains of transmission are unlikely under normal circumstances (11, 13). However, super spreading events, wherein a single infected individual transmits to an unusually large number of secondary cases, have been observed. Such events are influenced by factors including viral load, symptomatic presentation, and culturally specific practices around illness and death. The potential for viral adaptation to enhance human-to-human transmissibility remains a significant concern, as relatively minor genetic changes could potentially increase the R0 above the epidemic threshold of 1.0, leading to sustained transmission chains and larger outbreaks (11, 13).

ECOLOGICAL TRANSMISSION

Natural habitat destruction and land-use changes have disrupted bat foraging patterns; this has increased the frequency of bat-human interfaces. Specifically, increasing number of Pteropus bats has been seen to utilize agricultural area and human settlements for feeding and roosting (11). The migratory pattern of the bat due to climate change further compounds these effects, expanding their geographical range (Fig. 2), and potentially stressing bat populations in ways that may enhance viral shedding (14).

The establishment of large-scale pig farms and agricultural land in regions overlapping with flying fox habitats has created conditions conducive to viral amplification and human exposure (11). The Malaysian Nipah outbreak of 1998–1999 exemplifies this dynamic, wherein pig farms established beneath fruit trees frequented by bats created an ideal setting for virus introduction, amplification, and subsequent human infection (11). The practice of date palm sap collection in Bangladesh similarly represents an anthropogenic activity that creates a transmission pathway, as bats visiting the sap collection pots contaminate the sap with virus-containing saliva and urine, which is then consumed by humans without processing that would inactivate the virus (11).

Socioeconomic factors, like limited healthcare infrastructure, inadequate surveillance systems, and cultural practices surrounding caregiving and burial rituals can further facilitate viral spread once human infections occur (1, 11). Additionally, ecological encroachments, such as deforestation for agricultural expansion, create feedback loops that intensify spillover risk while constraining the resources available for prevention and response measures.

CLINICAL PROGRESSION

A variable course has been suggested for clinical progression, ranging from asymptomatic infection to severe fatal encephalitis, with distinctive pathophysiological mechanisms involving vascular damage, immune evasion, and potential for latent infection with delayed recrudescence.

INCUBATION PERIOD

Henipavirus infections typically manifest after an incubation period ranging from 4–14 days in humans, though in some rare cases it has been reported to extend up to 45 days (16). This variability in incubation period depends on multiple factors including viral dose, route of exposure, and host factors (17).

INITIAL PRESENTATION

Clinical manifestations resemble influenza-like illness initially, characterized by abrupt onset of fever, headache,

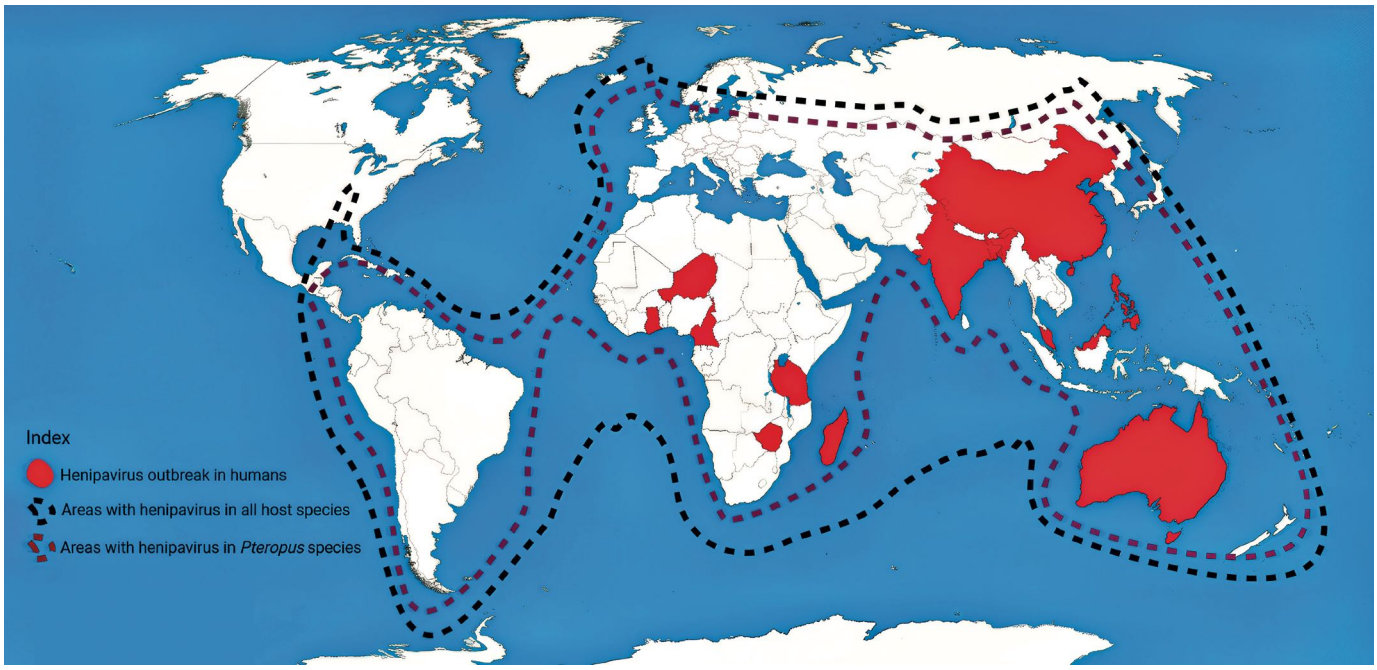

Fig. 2 Geographic distribution of regions inhabited by host species carrying henipavirus and human henipavirus outbreaks.

dizziness, and vomiting, typically followed by myalgia and general malaise (15). These non-specific prodromal symptoms make early diagnosis challenging, particularly in regions where other febrile illnesses such as malaria or dengue are endemic. The initial viral replication occurs in the respiratory epithelium before the virus disseminates systematically through endothelial cells entering the bloodstream (1).

Early phase respiratory symptoms include mild cough and sore throat, which can rapidly progress to more severe respiratory distress in some cases. The virus is known to be shed in nasal secretions even before the onset of symptoms, with evidence showing that horses can shed HeV in nasal secretions as early as two days post-exposure, prior to developing clinical signs (17). This pre-symptomatic shedding contributes to the transmission dynamics of these viruses and presents significant challenges for infection control during outbreaks. Laboratory findings typically shows non-specific changes such as leukopenia, thrombocytopenia, and elevated liver enzymes during the early clinical presentation, which lacks disease-specific pathognomonic features.

MECHANISMS OF TISSUE DAMAGE

The pathogenesis of henipavirus infection is linked to the virus's cellular tropism, which is determined by the distribution of its entry receptors, primarily ephrin-B2 and ephrin-B3 (8). These receptors are predominantly expressed in many tissues including endothelial cells, neurons, and respiratory epithelium; this explains the systemic nature of infection and the broad host range of henipaviruses compared to most other paramyxoviruses (19). This widespread receptor distribution facilitates viral dissemination to multiple organs and tissues throughout the body. Initially, direct viral damage to endothelial cells lining blood vessels, leads to a characteristic vasculitis observed in multiple organ systems.

A distinctive pathological feature of henipavirus infection is the formation of multinucleated giant cells known as syncytia (1). This occurs when viral glycoproteins expressed on the surface of infected cells bind to cellular receptors on neighboring cells, triggering membrane fusion mediated by the viral F protein (1). The resulting syncytia formation is associated with extensive tissue damage, including necrosis, vasculitis, and thrombosis in affected organs. Autopsy findings from NiV-infected patients have revealed widespread vasculitis in the lungs (62%), kidney (24%), heart (31%), and central nervous system (80%), correlating with the expression pattern of ephrin-B2 in these tissues (20). Additionally, necrosis is commonly observed in highly vascularized organs such as the spleen, particularly in regions expressing ephrin-B2 (1, 19). These pathological changes explain the clinical manifestations of respiratory disorders, neurological symptoms, and hemodynamic instability which are observed in henipavirus infections.

NEUROLOGICAL MANIFESTATIONS

Neurological manifestations become increasingly prominent when henipavirus infection progresses and often define the severe stage of disease. The virus can enter the central nervous system (CNS) through multiple routes: via infected endothelial cells of the blood-brain barrier, through direct infection of olfactory neurons from the nasal cavity, or via retrograde axonal transport (1, 21). Viral replication in the CNS leads to neuronal damage, inflammation, and disruption of the blood-brain barrier, resulting in progressive neurological impairment. Particularly in patients with reduced levels of consciousness, clinical neurological manifestations exhibit altered consciousness, areflexia, hypotonia, and/or abnormal doll's eye reflex (15, 20).

Neurological signs indicating acute encephalitis have been reported in more than 70% of cases, with severe weakness in 67% and areflexia or hyporeflexia in 65% (20). A distinctive and diagnostically significant finding seen in approximately 30% of NiV encephalitis patients is segmental myoclonus, which involves diaphragm and muscles in the limbs, neck, and face (20). Other neurological manifestations include meningism, generalized tonic-clonic seizures, nystagmus, and cerebellar signs, indicating the widespread involvement of different parts of the nervous system (22). In severe cases, progressive neurological deterioration leads to coma and death, with overall mortality rates ranging from 40% to 70% (21, 22).

RELAPSING AND LATE-ONSET ENCEPHALITIS

Perhaps one of the fascinating and clinically significant aspects of henipavirus infections is the occurrence of relapsing or late-onset encephalitis, which can develop weeks, months, or even years after the initial infection (21, 23). This phenomenon has been documented in both HeV and NiV infections, though it appears to be more common with NiV. Relapsing encephalitis may affect up to 10% of survivors and can occur following either symptomatic infections (ranging from mild illness to acute encephalitis) or even after asymptomatic seroconversion (15, 21). The most delayed case documented occurred 11 years after an asymptomatic infection, underscoring the potential for long-term viral persistence in the CNS (21).

Clinical and radiological observations indicate that encephalitis caused by recurring henipavirus infection presents differently from acute encephalitis (21). Magnetic resonance imaging in the relapsing phase typically reveals more extensive and confluent hyperintense cortical lesions compared to those documented during the acute phase. Pathologically, relapsing encephalitis is distinguished by widespread and confluent necrosis of the parenchyma, edema, and inflammation, primarily affecting neuronal regions. This condition is also characterized by prominent perivascular cuffing, severe loss of neurons, reactive gliosis, and neovascularization (15, 21). Viral inclusions, antigens, and RNA are predominantly detected in surviving neurons. In the relapsing form, the vasculitis, endothelial syncytia, and thrombosis typical of acute henipavirus encephalitis are absent. Furthermore, blood vessels do not contain viral antigens or RNA, suggesting that relapsing encephalitis results from the reactivation of latent viral foci within the central nervous system rather than reinfection or entry from outside the

CNS (21). The estimated case fatality rate for relapsed and late-onset Nipah virus encephalitis is approximately 18%, which is significantly lower than that of acute encephalitis (23).

DIAGNOSTIC

The diagnosis requires a combined clinical evaluation with laboratory confirmation. Clinical assessment should focus on identifying initial characteristic symptoms including encephalitis, fever, headache, respiratory distress, seizures, and altered mental status, particularly in patients with relevant exposure history in endemic regions (24). First-line diagnostic investigations include serology, RT-PCR, complete blood count, liver function tests, serum electrolytes, clotting profiles, chest radiography, and cerebrospinal fluid analysis (24, 25). For challenging cases, advanced imaging (MRI), electroencephalography, and rare brain biopsy may be necessary (24).

In the laboratory setting, diagnosis is made through several methods, with nucleic acid amplification testing (NAAT) serving as the gold-standard for definitive diagnosis due to its high sensitivity, detecting as few as 20 viral genomes (25). Reverse transcription polymerase chain reaction (RT-PCR) targets the conserved viral N, M, or P genome segments, can rapidly identify viral RNA in clinical specimens including throat and nasal swabs, cerebrospinal fluid, blood, urine, and respiratory secretions (25). RT-PCR demonstrates excellent sensitivity, detecting between 500–1000 copies of RNA templates with the lowest detection threshold at 0.37 pg/μL of RNA (24).

Enzyme-linked immunosorbent assay (ELISA) can detect both viral antigens and host antibody responses (IgM and IgG), typically requiring 3–4 hours for completion and applicable to both human and animal samples (24, 25). IgM antibody detection has been reported to peak approximately nine days after illness onset and can persist for at least three months, while IgG peaks after 17 days and remains detectable for more than eight months (25). Especially, in resource-limited settings where PCR facilities may be unavailable, serological testing complements molecular can be diagnostics. Recognition of the diagnostic challenges in rural and remote settings has prompted the development of point-of-care and “near-POC” NAAT platforms requiring minimal infrastructure and training, potentially tackling outbreak management capabilities in resource-limited areas (24).

MANAGEMENT

Henipavirus infections management remains predominantly supportive due to the absence of approved specific antiviral therapies, this presents significant challenges given the high mortality rates associated with these infections (24, 26). The primary treatment involves supportive care, focusing on fundamental clinical procedures: maintaining fluid and electrolyte balance, prophylaxis against venous thrombosis, ensuring airway patency, and mechanical ventilation for respiratory compromise (24). Broad-spectrum antibiotics are typically administered to prevent secondary bacterial infections that might complicate the clinical course (24). Ribavirin has been documented to yield promising results, reducing mortality by approximately 36%, when used empirically during the 1998–1999 Malaysian outbreaks (26). However, evidence remains limited due to the open-label nature of the studies and their reliance on historical controls. The broad-spectrum antiviral remdesivir demonstrated protection in African green monkeys when administered within 24 hours of NiV exposure and continued for 12 days (26).

Several experimental therapeutics shows encouraging potential for future treatment protocols. Favipiravir (T705) exhibited protective effects in Syrian golden hamsters, while chloroquine demonstrated efficacy in suppressing viral replication in cell cultures, though it’s in vivo efficacy remains uncertain (24, 26). Particularly promising are biologics such as the monoclonal antibody m102.4, which has demonstrated protection in animal models and has progressed to Phase I human trials with manageable adverse events, yielding encouraging results (24). Passive immunotherapy approaches using monoclonal antibodies have protected ferrets against NiV infection and hamsters from HeV (27). Novel compounds such as Griffithsin (GRFT) and its synthetic derivatives have demonstrated significant inhibition of viral replication, with in vivo evaluations in golden Syrian hamsters showing protection against lethal NiV challenge (24). Although progress has been made, the treatment window remains narrow, usually within 24 hours of exposure, emphasizing the critical need for rapid diagnostics and accessible treatments to improve survival rates (26). For patients who recover, long-term follow-up remains essential due to the potential for relapsing encephalitis and neurological sequelae.

CONCLUSION

The recent discovery of CHV in North America represents a significant expansion in our understanding of henipavirus distribution globally. This finding demonstrates that these potentially deadly pathogens are not confined to their previously known ranges in Asia, Australia, and Africa, but may be more widely distributed throughout the world.

The high fatality rates associated with henipaviruses like Nipah and Hendra emphasizes the importance of proactive research and preparedness efforts. Public health systems should remain vigilant for unusual disease patterns, particularly in regions where known intermittent hosts are common.

As humans continue to encroach on wildlife habitats and climate change alters ecological relationships, the emergence of novel pathogens becomes increasingly likely. The CHV discovery serves as a timely reminder of the ongoing need for robust disease surveillance systems and coordinated international responses to emerging infectious disease threats before they develop into epidemic or possible pandemic.

AUTHORS' CONTRIBUTIONS

Biplab Adhikari: Conceptualization, data acquisition, formal analysis, supervision, drafting the original manuscript, creating diagrams and figures, reviewing, and editing and submission of final version.

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Treatment of Chondral Defect of the Knee Joint – Current Methods, Possibilities of Using Cultured Mesenchymal Stem Cells

Libor Prokeš[1,*], Tomáš Kučera[1]

ABSTRACT

Cartilage damage is caused by degenerative process and also by trauma, microtrauma or as a consequence of avascular necrosis. The damage may be focal or diffuse over a larger area. Because of the limited healing potential, treatment of articular cartilage injuries is problematic. The choice of surgical treatment depends on several factors: the size, depth and location of the defect, the age of the patient, the desired activity, associated changes and the possibility of postoperative rehabilitation. Finding an appropriate method of treatment for chondral defects with a reliable long-term outcome is difficult. The most common, clinically proven and used surgical techniques include abrasive chondroplasty, marrow stimulating techniques, transplantation procedures or a combination of methods. The possibility of introducing new methods in the form of the application of cultured mesenchymal stem cells represents a significant advance in the field of regenerative medicine. Their use is safe and effective.

KEYWORDS

chondral defect; mesenchymal stem cells; 3D carrier; hyaline cartilage

AUTHOR AFFILIATIONS

[1] Department of Orthopedy, University Hospital and Charles University, Faculty of Medicine, Hradec Králové, Czech Republic

* Corresponding author: Department of Orthopedy, University Hospital and Charles University, Faculty of Medicine, Sokolská 581, 500 05 Hradec Králové, Czech Republic; libor.prokes@fnhk.cz

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INTRODUCTION

Cartilage is a connective tissue composed of chondrocytes and extracellular matrix with collagen fibrils, it has a firm consistency and its property is compressive elasticity. It responds to pressure by deforming its shape, which returns to its original form when the pressure subsides. The proteoglycans contained in the extracellular matrix and the collagen fibrils are responsible for this elasticity. The cartilage is vascular-free and nutrition is provided by diffusion through the intercellular matrix. Cartilage function depends on the quantitative and qualitative ratio of proteoglycans and their glycosaminoglycan (GAG) chains and the arrangement of collagen fibrils. Articular cartilage acts as a shock absorber and ensures an even distribution of shock to the surface (1).

Cartilage damage is caused by degenerative process and also by trauma, microtrauma or as a consequence of avascular necrosis. The damage may be focal or diffuse over a larger area. Degenerative changes in cartilage are characterized by damage to collagen fibres and loss of GAG chains; the ratio of individual particles changes with an increase in water content. Due to its limited regenerative capacity, any damage to cartilage is a serious problem. Its main disadvantage is the limited ability of the internal repair processes of its own chondrocytes, which are rigidly fixed in the protein matrix. Therefore, they cannot migrate to the injured area and participate in the repair process. Patients with a deep chondral defect suffer from pain, swelling and limitation of joint mobility. Untreated cartilaginous lesions gradually progress and lead to the development of premature arthrosis.

The aim of this thesis is to present current modern methods of treatment of post-traumatic and degenerative chondral defects with the possibility of using cultured bone marrow mesenchymal stem cells.

IMAGING METHODS

X-RAY IMAGE

Among the radiodiagnostic methods, the classical X-ray examination takes the leading position. However, normal hyaline cartilage is not contrasted on sciagraphic examination, and therefore we evaluate it indirectly by the spread of the articular cleft and the reaction of the surrounding bone (subchondral sclerotization, reactive bidding and the formation of osteophytes of the articular surfaces marginally). It is of major importance for the detection of osteochondral fragments.

MAGNETIC RESONANCE

Magnetic resonance imaging holds a dominant position among the examination methods of articular cartilage pathologies. The development of examination techniques has optimized the display of cartilage morphology, its volumetry and now also the possibility of biochemical analysis. Due to its high resolution and spatial resolution, it is an ideal method for imaging the soft tissues of the joint and cartilage in its entirety, including the pathologies present. Another advantage is the possibility of volumetric measurement of cartilage when assessing the progression of degenerative and inflammatory diseases or monitoring during therapy, both osteochondral graft attachment and medical therapy. Its indisputable advantage is that its non-invasiveness does not burden the patient. Another advantage of the newer MRI machines is the use of sequences with excellent resolution of cartilaginous tissue. According to the information available in the literature, the authors have the most experience and achieve good results in imaging chondral pathologies using proton density (PD), T2 Fast Spin Echo (T2 FSE) or Fast LowAngle-SHot (FLASH) sequences. Fat signal suppression sequences have become useful for imaging cartilage pathologies. PD and T2 FSE sequences are advantageous for visualizing damage to the middle and deep layers of cartilage, while the FLASH sequence is useful for visualizing superficial lesions (2, 3). Furthermore, the author's experience shows the usefulness of multiplanar imaging, which increases sensitivity and specificity in detecting chondral lesions (3–6). Newer methods such as T1rho and T2 mapping, Na+ imaging or delayed cartilage saturation methods such as dGEMRIC (delayed Gadolinium-Enhanced) are gaining importace.

TREATMENT OF CHONDRAL DEFECTS

Because of the limited healing potential, treatment of articular cartilage injuries is problematic. The choice of surgical treatment depends on several factors: the size, depth and location of the defect, the age of the patient, the activity required, associated changes and the possibility of postoperative rehabilitation. The development of arthroscopy with the development of more sophisticated instrumentation and with it new surgical techniques has been a shift in the possibility of treating and diagnosing not only chondral lesions. Finding a suitable method of treatment for chondral defects with reliable long-term results is difficult.

Joint resurfacing surgery alone may be without effect if the stability and axis of the limb is not restored. Failure of the limb axis leads to excessive pressure on the contact surface. This condition can be influenced by an appropriate type of osteotomy. The main aim of proximal tibial osteotomy is to improve the biomechanical aspects and biological properties of the joint. The horizontal positioning of the articular cleft and the correction of the mechanical axis by a slight re-alignment to valgus leads to a shift of the loading force from the damaged compartment to the undamaged one. The blood supply increases in the vicinity of the osteotomy and the rate of venostasis decreases in the damaged parts of the joint. The change in innervation is usually associated with a decrease in pain and indirectly with an increased range of motion in the joint. When the indication criteria and methodology are followed correctly, corrective osteotomy contributes to a reduction in pain and slows the progression of arthrosis (7). Regeneration of the hyaline cartilage of the medial compartment of the knee during "second look arthroscopy" has also been described by some authors (8). A less common deformity is valgus

deformity, for which a variation osteotomy of the lower femur is recommended. Similarly, joint stability is a concern. Surgical procedures that stabilize the knee joint, such as cruciate ligament repairs, reduce the risk of developing a cartilage defect and the risk of developing arthrosis.

The most common, clinically proven and used surgical techniques include abrasive chondroplasty, marrow stimulating techniques, transplantation procedures or a combination of methods. Abrasion chondroplasty (9) is used for chondral defects of smaller size (up to 2 cm^2) or in patients with osteoarthritis. The cartilage defect is aligned and the unstable edges of the defect are removed to restore a smooth surface and remove the delaminated portions. Abrasion of the base of the chondral defect does not extend below the zone of calcified cartilage. Marrow-stimulating techniques consist of perforating the subchondral bone after removal of the remnants of damaged cartilage, allowing migration of undifferentiated mesenchymal stem cells into the defect area and vascular ingrowth. The first results of this technique were published by Pridie (10) in 1959, when he performed retreatments into the base of a cleaned defect and by Ficat (11). Over time, the designs were replaced by Steadman microfractures (12), which is currently the most widely used marrow-stimulating technique applicable not only in the knee (Fig. 1) but also in the shoulder (13) and hock (14) joints. A special curved chisel is used to break the subchondral bone 4 mm deep, 5 mm apart. The blood forms a fibrin plug at the base which starts to stimulate tissue healing. After 8–12 weeks, the primary plug transforms into fibrocartilaginous tissue. The fibrous reparative tissue formed is less robust and less resistant than the original hyaline type of cartilage. The effect of this surgical technique is less, especially in patients over 40 years of age and in those where the defect is larger than 2.5 cm^2. In the long term, it does not prevent the onset and progression of degenerative changes. Another group of surgical procedures are transplantation procedures including implantation of cultured autologous chondrocytes, implantation of allogeneic osteochondral graft, transplantation of autologous osteochondral autografts (mosaicplasty) and implantation of structural supports. Transplantation of cultured autologous chondrocytes (ACI) is indicated for circumscribed deep chondral and osteochondral defects of the weight-bearing joints in younger patients ideally under 40 years of age within 3 cm^2. The first results of the use of cultured chondrocytes were published by Peterson in 1984 (15). In 1994, Brittberg (16) published the first results of implantation of a suspension of cultured chondrocytes fixed with a periosteal flap sutured to the articular surface. The results of the ACI method were very good, yet this method had its drawbacks in the form of a two-stage procedure, laborious suturing of the periosteal flap, damage or loosening of the flap with spillage of the suspension, or hypertrophy of the flap. Therefore, new fixation options have been gradually developed with the emergence of new generations of carriers (tissue glue, collagen membranes, hyaluronic acid esters, polylactide). The carrier must be biocompatible and biodegradace and must meet the conditions of good adhesion of chondrocytes with their dispersion in different polymer matrices already during in vitro production. This third-generation ACI technique, referred to as MACI (Matrix Induced Autologous Chondrocytes Implantation), has spread rapidly with very good results (17). Clinically used implants include polylactide acid polymer carriers (BioSeed-C), hyaluronic acid (Hyalograft-C) and autologous chondrocytes fixed in fibrin (Chondrograft). The results of the histological examination showed the formation of a mixture of connective and hyaline cartilage, collagen type II and proteoglycans. The resulting tissue is softer, well integrated with the subchondral bone. Stiffness tests lead to the conclusion that it is a regenerate of connective cartilage. Despite this fact, good medium-term clinical results are achieved, with a significant reduction in knee pain within one year and an improvement in joint function. Another method is the transplantation of autologous osteochondral grafts (mosaicplasty) described by Hangody (18, 19), designed to treat defects of 2–3 cm^2. The principle is to take cylindrical osteochondral blocks from the nonload zone of the joint and transfer them to the defect site after preparation of the bone bed (Fig. 2). The bone component integrates well with the surrounding bone. Hyaline cartilage maintains its properties and adds congruence to the articular surface. The defect is 70% covered by hyaline cartilage, with connective tissue between the blocks. The disadvantage of mosaicplasty is the transfer of tissue that has different biomechanical properties to a site with different load requirements. Another disadvantage is the risk of healing failure at the donor site, referred to in English literature as “donor site morbidity”. In mosaic plastics, it is reported that up to 50% of patients have non-specific joint discomfort from the donor site. This could be eliminated by sizing the harvest block below the so-called critical defect size of 7 mm. Ligamentous cartilage always develops at the harvest zone. With the correct surgical procedure, the method works very well, it is a one-time and economically inexpensive method. An alternative to this method is the implantation of freshly frozen osteochondral allografts. Allogeneic grafts are mainly used to treat large osteochondral defects (6 to 8 cm^2 in diameter) for indications of traumatic cartilage lesions, osteonecrosis or osteochondrosis dissecans. The disadvantages of this method are the risk of an immune reaction to the graft and the risk of disease transmission to the recipient. Currently, the most popular method is the combination of abrasive techniques with the implantation of biocompatible, bioconductive materials, which are in liquid, gel or solid cross-linked polymer form (3D carriers). The essence of the AMIC (Autologous Matrix-Induced Chondrogenesis) technique is to implant a carrier that promotes the ingrowth of mesenchymal stem cells and their differentiation into a chondrogenic line after microfracture into the base of the defect, thereby promoting the formation of new cartilaginous tissue (20, 21). The biomaterial forms a hemostatic barrier, therefore no bleeding into the joint cavity occurs. The implant is resorbed within approximately 40–60 days after implantation. It is usually fixed to the subchondral bone using biodegradable nails or tissue glue. AMIC therapy has given better results than microfracture alone in prospective studies (22). The AMIC technique is safe, functional, and effective for small to moderate (2 × 3 cm) cartilage defects, particularly in the knee. Some authors use the method for defects up to 8 cm^2 (23). The most commonly used polymeric materials include

Chondrotissue® (polyglycolic acid-based implant), Hyalofast® (esterified hyaluronic acid), Novocart® (type I collagen). Another option is artificial implants functioning as inserts (hydroxyapatite crystals with type I collagen).

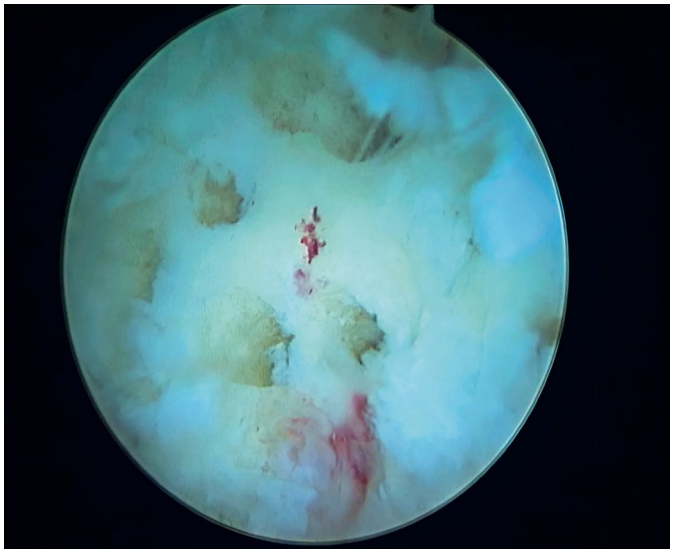

Fig. 1 Microfracture Steadman.

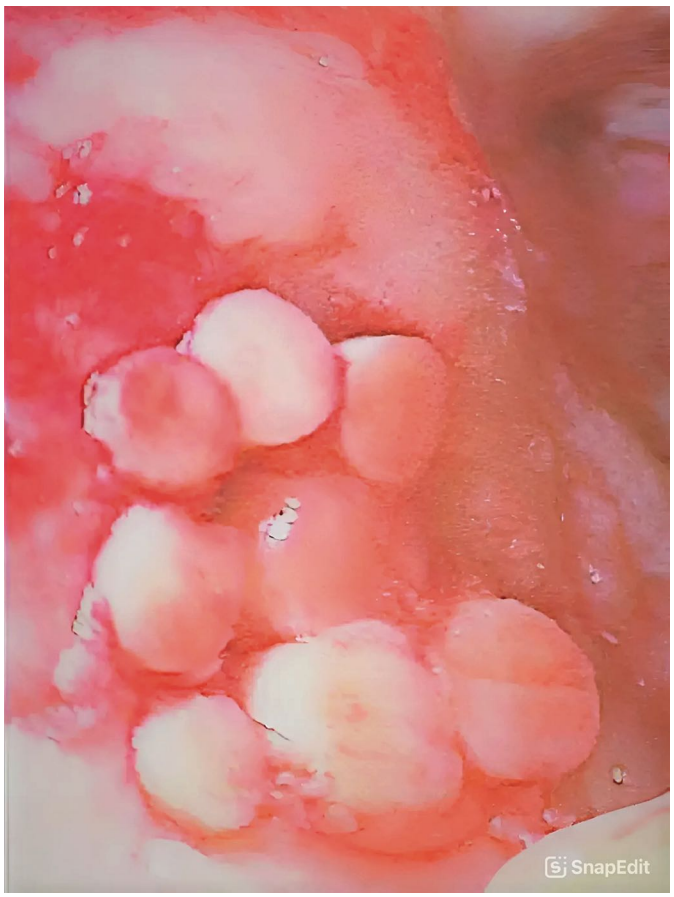

Fig. 2 Mosaicplasty.

We are currently conducting a clinical study in our clinic to help us refine this method using cultured mesenchymal stem cells. Mesenchymal Stem Cells (MSC) are multipotent stem cells that have the ability to differentiate into different cell types such as osteocytes, chondrocytesand adipocytes. MSCs are naturally found in various tissues, including bone marrow (the main source of MSCs), adipose tissue, synovial fluid etc. The basic properties of stem cells include:

1. Multipotency: the ability to differentiate into different cell types.
2. Immunomodulatory properties: MSCs can regulate the immune response, which is important for the treatment of inflammatory diseases.
3. Paracrine effect: they produce various bioactive molecules that promote tissue regeneration and angiogenesis (formation of new blood vessels).
4. Easy insulation and expansion: MSC can be easily obtained and propagated under laboratory conditions.

Mesenchymal stem cells have been extensively researched in the field of regenerative medicine due to their ability to repair damaged tissues. In recent years, MSC research has focused on thein use in clinical practice. Many clinical trials are underway to investigate their safety and efficacy in various diseases. Importantly for use in clinical practice, they are not teratogenic and can be used both autologous and allogeneic. Mesenchymal stem cells have achieved good results in in vitro tests (24), in animal models (25) and in early clinical trials in humans (26, 27). Bone marrow-derived stem cells have been shown to have a higher capacity for chondrogenic differentiation than adipose tissue-derived stem cells (28). Bone marrow-derived MSCs also produce significantly more type II collagen and glycosaminoglycan than adipose-derived stem cells (29). Although the results are promising, MSC therapy still faces challenges such as standardizing their isolation and culture, identifying the ideal dose, and long-term safety. At our institution, we obtain MSCs from the hip flap by biopsy needle harvesting of 26–30 ml of aspirate under local anesthesia 3–4 weeks before surgery. After isolation and expansion of the MSCs, the surgical procedure is performed, which consists of a mini-arthrotomy, removal of the malar cartilage remnants, alignment of the edges of the defect and treatment of its fundus with Priedi flaps (Fig. 3). Subsequently, a cell suspension - BiCure®orthoMSCp is uniformly applied to the 3D Hyalofast carrier in the operating room before final treatment of the carrier, resulting in a concentration of 0.98 (± 0.19) × 106 cells per cm^2 of carrier surface. After the gelation phase, the carrier is adjusted to the desired size and shape. After preparation, it is implanted into the defect site in one or 2 layers and fixed using Tisseel fibrin sealant (Fig. 4). After testing the primary stability by movement, a wound suture is performed without the use of a drain. Postoperatively, fixation of the knee in the brace is rigid for 2 days, followed by 0–60 degrees for 2 weeks, then 0–90 for 4 weeks. After six weeks, full loading of the knee joint is gradually allowed.

DISCUSSION

The above methods of defect treatment produce good results in most cases. Finding a suitable method of chondral defect treatment with reliable long-term results is diffi-

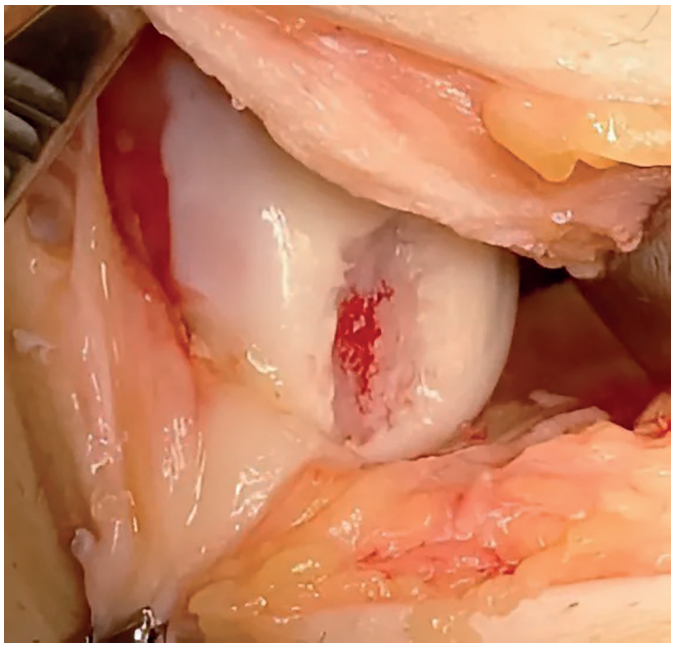

Fig. 3 Pridie technique.

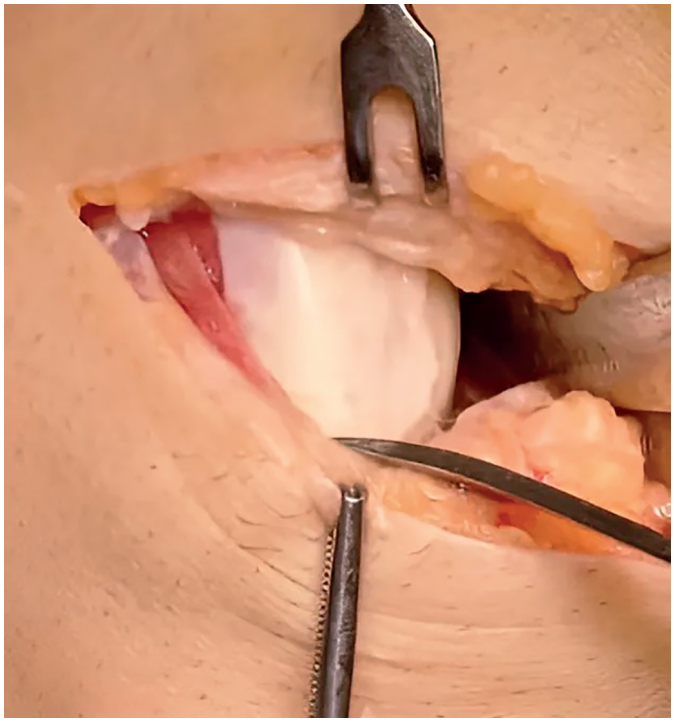

Fig. 4 Status after MSC carrier implantation.

cult. It is not possible to determine exactly which procedure is better for a particular type of defect, age of the patient, etc. Microfractures of the base of the defect are considered the gold standard in the treatment of chondral defects, although they have their limitations. The ligamentous repair tissue created is less robust and less resistant than the original hyaline type of cartilage (30). The effect of this surgical technique is less, especially in patients over 40 years of age and in those where the defect is larger than 2.5 cm^2. In the long term, it does not prevent the onset and progression of degenerative changes. The currently available systematic analyses of clinical results indicate an effective improvement in knee function in the first 2 years after microfracture, after which there is a gradual deterioration (31). To improve the results, the technique of microfracture of the base of the defect with implantation of a carrier that promotes the ingrowth of mesenchymal stem cells and their differentiation into a chondrogenic line has been used, thereby contributing to the formation of new cartilaginous tissue (20, 21). The results of AMIC therapy give better results than microfracture alone in prospective studies (22). The AMIC method is safe, functional and effective for small to moderate (2 × 3 cm) cartilage defects, especially in the knee. Most clinical studies report that cell therapy with autologous chondrocytes gives equal or better results than mosaicplasty (32). Transplantation of cultured autologous chondrocytes (ACIs) is indicated for circumscribed deep chondral and osteochondral defects of the weight-bearing joints in younger patients ideally under 40 years of age within 3 cm^2. Histological examination results showed the formation of a mixture of fibrous and hyaline cartilage and type II collagen and proteoglycans were demonstrated. The resulting tissue is softer, well integrated with the subchondral bone (33). The disadvantages of ACI remain its economic cost, the two-step surgical procedure and the harvesting of healthy cartilage, which entails pain from the donor tissue harvest site in up to 15% of patients (34). Another problem is the difficulty of in vitro expansion of chondrocytes and the difficulty of culturing with preservation of the quality and quantity of the harvested and subsequently implanted cells. In contrast, the use of cultured mesenchymal stem cells has the advantages of less invasive harvesting under local anaesthesia, one surgical procedure on the affected joint. MSCs also have a higher proliferative capacity than chondrocytes, which does not decrease significantly with patient age. Donor age and sex do not significantly affect the expansion capacity of MSCs. At our department, we perform bone marrow aspirate collection from the iliac bone flap. In their dissertation, the authors (35) perform quantitative and qualitative analysis of bone marrow cells from different sampling sites. The concentration of MNCs (MonoNuclear Cells) was significantly higher in the bone marrow aspirate taken from the hip bone flap. The median number of MNCs obtained from the tibia after sedimentation was 5.4 × 106 MNCs/ml, whereas 20.5 × 106 MNCs/ml were obtained from the hip bone flap. The measurement showed statistical significance and a greater proportion of MSCs to MNCs originating from the hip flap (5.2%), whereas the proportion was only 1.0% for the knee. Qualitative analysis focusing on immunophenotyping, viability, yielded comparable results for both sampling sites. The 3D Hyalofast carrier used in our study provides support to the implanted stem cells and allows their incorporation into the surrounding tissue and, conversely, cells from the surrounding cartilage and subchondral bone into the implant.

CONCLUSION

Current surgical methods of treating damaged cartilage have very good results according to studies and our experience. The possibility of introducing new methods in

the form of the application of cultured mesenchymal stem cells represents a significant advance in the field of regenerative medicine.

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Clinical Evaluation of Oxidized Cellulose Powder and Endoscopic Applicator in Multicenter Trial

Petr Habal[1], Veronika Sívková[2,*], Zdeněk Šorm[1], Milan Chobola[3], Jiří Feix[3], Igor Slaninka[3], Jiřina Habalová[4], Tomáš Hosszú[4], Jaroslav Pacovský[5]

ABSTRACT

Purpose: Oxidized cellulose-based haemostatic agents are widely used for managing bleeding in various surgical procedures. This study evaluates the efficacy and safety of oxidized cellulose powder and an endoscopic applicator across a broad spectrum of surgical settings.
Methods: This was a prospective, multicentre study involving 99 evaluable patients undergoing surgeries with varying bleeding severities and surgical approaches (open, laparoscopic, or thoracoscopic). The primary endpoint was achieving haemostasis within 3 minutes and avoiding revision surgery within 12 hours. The time to haemostasis (TTH) and complications were recorded, and statistical comparisons were made using a paired and unpaired t-test, with a significance threshold of $P < 0.05$. Data from this study were compared to historical results from fibrillar haemostats.
Results: Haemostasis was achieved within 3 minutes in 61.6% (95% CI [52.0, 71.2]) of patients and within 5 minutes in 99.0% (95% CI [97.0, 100.0]) of patients. The overall mean TTH was 153.8 seconds (95% CI: 141.5–166.1), with shorter TTH observed in minimally invasive procedures using the endoscopic applicator. Subgroup analysis revealed higher success rates for patients with mild bleeding (78%) compared to moderate bleeding (50%).
Conclusion: Oxidized cellulose powder demonstrates reliable haemostatic performance across diverse surgical applications. The endoscopic applicator enhances precision and applicability, particularly in minimally invasive settings, making it a valuable tool in modern surgical practice.

KEYWORDS

oxidized cellulose; haemostasis; endoscopic applicator; haemostatic powder; Traumastem

AUTHORS AFFILIATIONS

[1] Department of Cardiac Surgery, Charles University, Faculty of Medicine and University Hospital in Hradec Králové, Czech Republic
[2] BIOSTER, a. s.
[3] Department of Surgery, Charles University, Faculty of Medicine and University Hospital in Hradec Králové, Czech Republic
[4] Department of Neurosurgery, Charles University, Faculty of Medicine and University Hospital in Hradec Králové, Czech Republic
[5] Department of Urology, Charles University, Faculty of Medicine and University Hospital in Hradec Králové, Czech Republic
* Corresponding author: Tejny 621, 664 71 Veverská Bítýška, Czech Republic; v.sivkova@bioster.cz

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INTRODUCTION

The ability to achieve effective haemostasis during surgical procedures is critical for minimizing blood loss, ensuring clear surgical fields, and reducing postoperative complications. Despite advancements in surgical techniques, capillary and diffuse bleeding remain common challenges that may be in procedures involving parenchymal organs, vascular anastomoses, or delicate tissue handling. Conventional methods, such as sutures, ligatures, and electrocautery, often provide effective control but may fail in cases of diffuse oozing from capillary beds or in hard-to-reach anatomical locations. Local haemostatic agents have emerged as indispensable tools for addressing these challenges (1, 2). Among these, oxidized cellulose-based products offer unique advantages. Oxidized cellulose is a naturally derived polysaccharide that has been widely utilized in medical applications, particularly as a haemostatic agent that is biodegradable, bioresorbable, and biocompatible, making it an ideal choice for surgical interventions (3). The secondary effect is bactericidal and antifungal, driven by a low pH, which eliminates microorganisms and prevents their growth and proliferation (2). Oxidized cellulose-based haemostatic agents have a history spanning nearly ninety years. Initially developed in the United States to advance surgical techniques in both military and civilian medicine, their method of preparation was first documented in 1945 (3).

Traumacel PULVIS, (other names: Traumastem POWDER, EMOXICEL EMIPOL, Resorcell) developed in the 1970s in Czechoslovakia, has been extensively used in surgical practice for decades. Its broad applicability includes use in open surgeries, minimally invasive procedures, and even outpatient settings for minor injuries (4). This powder containing a hydrogen calcium salt of oxidized cellulose, is a specialized haemostatic agent that leverages the synergistic effects of oxidized cellulose and calcium ions to enhance haemostasis and promote wound healing. Oxidized cellulose stops bleeding through a combination of physical absorption, gel formation, platelet activation, and stabilization of the clot. Studies have shown that the activation of blood platelets by oxidized cellulose, depends on the availability of calcium ions in its dispersion (5).

Beyond its role in coagulation, the hydrogen calcium salt of oxidized cellulose has demonstrated significant healing properties due to the combined action of oxidized cellulose (OC) and calcium ions. This combination has been shown to enhance fibroblast proliferation, as evidenced by an in-vitro study conducted by Hughes. This property accelerates the formation of granulation tissue, leading to faster wound healing (6). Another research highlights the unique healing effects of the hydrogen calcium salt of OC (7). The combination of OC and calcium ions not only facilitates haemostasis but also actively supports the biological processes involved in tissue repair and regeneration.

The synergistic effects of OC and calcium ions are well-documented in numerous publications. However, the number of publications on the effectiveness of OC-based haemostatic powder remains limited compared to the extensive research on haemostatic gauze and fibrillar forms.

METHODS

OBJECTIVES

The primary objective of the study was to confirm the safety and effectiveness of the haemostatic agent Traumacel PULVIS, (OC powder) and the endoscopic applicator Traumacel ENDO Applicator in real-world surgical settings. By evaluating these devices across a diverse patient population, this research aims to provide robust data supporting their continued use in modern surgical practice. The results were compared with primary data from a published clinical study of a similar design that evaluated OC fibrillar haemostats (8).

STUDY DESIGN

This prospective, multicentre clinical study was conducted as part of post-market clinical follow-up (PMCF), ensuring rigorous scientific and ethical oversight. The single-arm design included patients undergoing various surgical procedures across four centres in the Czech Republic. The study centres represented a range of surgical disciplines, including thoracic, cardiovascular, general, and urologic surgery, ensuring a broad evaluation of the devices.

An achievement of haemostasis within three minutes and avoidance of revision surgery within 12 hours were set as primary endpoint. Time to Haemostasis (TTH), degree of bleeding at the application site and incidence of adverse events (AEs) and postoperative complications were observed.

Data were collected using a standardized Case Report Form (CRF), which captured detailed intraoperative and postoperative observations.

ETHICAL ISSUES

This study was conducted in accordance with the Declaration of Helsinki (52nd WMA General Assembly, Edinburgh, Scotland, October 2000) and was approved by the Ethics Committee of the University Hospital Hradec Králové, Czech Republic. Written informed consent was obtained from all participants, who were also given the option to withdraw from the study at any time without obligation or penalty.

PATIENT POPULATION

A total of 100 patients were screened for inclusion, with 99 ultimately enrolled in the per-protocol set (PPS) after exclusions. The study population consisted of adult patients undergoing open, laparoscopic, thoracoscopic, or robotic surgeries. Demographics, including age, gender, and comorbid conditions, were recorded to provide context for efficacy and safety outcomes. Inclusion criteria were age above 18 years or older and capillary or diffuse bleeding unmanageable by conventional methods. Exclusion Criteria were known hypersensitivity to oxidized cellulose, pregnancy or severe medical conditions compromising safety. If another local haemostatic agent was used, patients were excluded from the effectiveness assessment (PPS) but were included in the Safety Set (SS).

USED HAEMOSTATS

Traumacel PULVIS (other names: Traumastem POWDER, EMOXICEL EMIPOL, Resorcell; BIOSTER, a. s., Veverská Bítýška, Czech Republic) is a sterile, bioresorbable powder designed to achieve haemostasis across various surgical fields. It is packaged in 1, 2, 3, and 5 g doses, with the 2 g package utilized in this study. An applicator Traumacel ENDO Applicator, (other names: Traumastem ENDO Applicator, EMOXICEL ENDO Applicator; BIOSTER, a. s., Veverská Bítýška, Czech Republic) was used for endoscopic procedures or in hard-to-reach areas. This applicator specifically designed for laparoscopic and thoracoscopic surgeries, allows precise application in minimally invasive settings, extending the versatility of the product.

STATISTICAL ANALYSIS

The safety analysis included all patients who received the tested haemostat (Safety set – SS), while the efficacy analysis was conducted on patients who adhered to the study protocol without deviations. (Per Protocol Set – PPS) The proportion of patients meeting the primary endpoint and the mean time to haemostasis (TTH), both with 95% confidence intervals, were evaluated. In addition, subgroup analyses were performed based on bleeding severity and surgical approach. Mean TTH across the total groups was compared using an unpaired t-test, with statistical significance defined as $P < 0.05$.

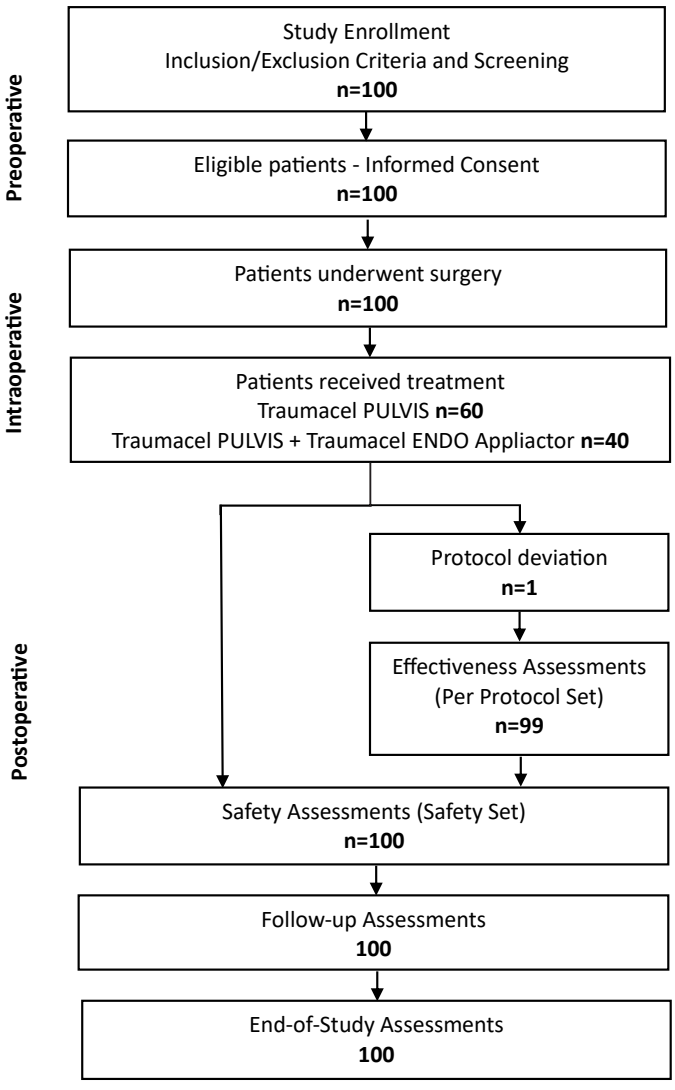

Fig. 1 Study Flowchart.

RESULTS

DEMOGRAPHICS AND PATIENT CHARACTERISTICS

The study enrolled a total of 100 patients across four surgical centres, with 99 included in the per-protocol set (PPS) after one exclusion due to protocol deviation.

The mean age of patients was 60.8 years, with 60.6% male and 39.4% female participants.

A substantial portion of the study population (42.4%) had significant comorbidities, including diabetes mellitus, ischemic heart disease, and oncologic conditions, which could potentially influence surgical outcomes.

The inclusion of patients with diverse comorbidities ensured that the results reflected real-world clinical scenarios.

The thoracic region was the most frequent target bleeding site (TBS), representing 50% of cases, followed by retroperitoneal/abdominal (21.0%) and cutaneous/subcutaneous sites (10.0%). Notably, in 55% of the cases, no additional methods were required to control bleeding beyond the application of the OC powder, with electrocoagulation being the most frequently used adjunctive technique (34%). These findings highlight the versatility and effectiveness of the OC powder across a variety of surgical procedures and bleeding scenarios.

PRIMARY OUTCOMES

A total of 61.6% (95% CI [52.0, 71.2]) of patients achieved haemostasis within three minutes after the OC powder application, meeting the primary efficacy endpoint. One patient did not meet the primary endpoint due to revision surgery within 12 hours postoperatively. However, the revision was due to a mechanical failure of a vein graft ligature, unrelated to the performance of tested product.

Tab. 1 Demographic Characteristics (PPS).

Characteristic	Traumacel PULVIS n (%) N = 60 (PPS)	Traumacel PULVIS + Traumacel ENDO Applicator n (%) N = 39 (PPS)	Total n (%) N = 99 (PPS)
Age, mean (SD)	62.0 (16.41)	58.9 (17.09)	60.8 (16.58)
Age, MIN – MAX	18–88	20–83	18–88
Sex, n (%)			
Male	35.0 (58.3)	25.0 (64.1)	60.0 (60.6)
Female	25.0 (41.7)	14.0 (35.9)	39.0 (39.4)
PHCCM, n (%)	21.0 (35.0)	21.0 (53.8)	42.0 (42.4)

SD, standard deviation; PHCCM, possible health complications, comorbidities and medications that may affect the outcome of treatment; PPS, Per Protocol Set (for more information, see Table 2).

Tab. 2 List of PHCCM and occurrence.

	Occurrence
Comorbidities	Oncologic disease (5), ischemic heart disease (4), diabetes mellitus (1), arterial hypertension (5), renal failure (3), Ischemia of lower extremities (1), hypothyroidism (1)
Medications	Acetylsalicylic acid (15), warfarin (6), heparin and its derivatives (6), apixaban (1), ticagrelor (1), corticosteroids (3), diosmin (1), dabigatran (1), clopidogrel (1), chemotherapy (2)
Other complications	smoking (5), alcohol abuse (1), obesity (2), condition after kidney transplantation (1), condition after kidney resection (1), immobility (1), warfarin discontinued (1), anopyrin discontinued (3), dialysis (1)

PHCCM, possible health complications, comorbidities and medications that may affect the outcome of treatment.

Tab. 3 Operative procedures (SS).

Parametr	Traumacel PULVIS n (%) N = 60	Traumacel PULVIS + Traumacel ENDO Applicator n (%) N = 40	Total n (%) N = 100
Number of Patients Screened	60 (60.0)	40 (40.0)	100 (100)
Did Not Meet Preoperative Eligibility	0 (0.0)	0 (0.0)	0 (0.0)
Did Not Meet Intraoperative Eligibility	0 (0.0)	1 (1.0)	1 (1.0)
Type of intervention			
Classic	60 (100.0)	11(27.5)	71 (71.0)
Laparoscopic	0 (0.0)	13 (32.5)	13 (13.0)
Thoracoscopic	0 (0.0)	16 (40.0)	16 (16.0)
Robotic	0	0	0
Endoscopic	0	0	0
Anatomic location of TBS, n (%)			
Thoracic	27 (45.0)	23 (57.5)	50 (50.0)
Retroperitoneal / Abdominal	10 (16.7)	11 (27.5)	21 (21.0)
Pelvic	5 (8.3)	2 (5.0)	7 (7.0)
Cutaneous / Subcutaneous	9 (15.0)	1 (2.5)	10 (10.0)
Extremities	7 (11.7)	0 (0.0)	7 (7.0)
Spinal canal	2 (3.3)	3 (7.5)	5 (5.0)
Other methods used to stop bleeding from TBS, n			
Electrocoagulation only	21 (35.0)	13 (32.5)	34 (34.0)
Mechanical methods only	5 (8.3)	0 (0.0)	5 (5.0)
Pharmacological methods only	2 (3.3)	0 (0.0)	2 (2.0)
Electrocoagulation + mechanical methods	2 (3.3)	1 (2.5)	3 (3.0)
Electrocoagulation + pharmacological methods	1 (1.7)	0 (0.0)	1 (1.0)
None	29 (48.4)	26 (65.0)	55 (55.0)

SS, Safety set.

The other patients achieved haemostasis within 5 minutes. The proportion of patients with haemostasis within 5 minutes without surgical revisions in 12 hours was 99.0% (95% CI [97.0, 100.0]).

The primary outcomes of the study were analysed across different subgroups, categorized by bleeding severity (mild, moderate, or severe) and type of surgery (open or endoscopic). Among patients with mild bleeding 78% (95% CI [66.5, 89.5]) achieved the primary outcome. In the group of patients with moderate bleeding 50% (95% CI [35.6, 64.5]) of patients met the primary outcome. None of the patients with severe bleeding achieved the primary outcome. In terms of surgical approach, 59.2% (95% CI [47.7, 70.6]) of patients undergoing open surgery achieved the primary outcome. For endoscopic procedures, the success rate was higher, at 67.9% (95% CI [50.6, 85.2]). These findings indicate variability in outcomes depending on the severity of bleeding and the type of surgical intervention, highlighting the importance of patient-specific factors in achieving effective results.

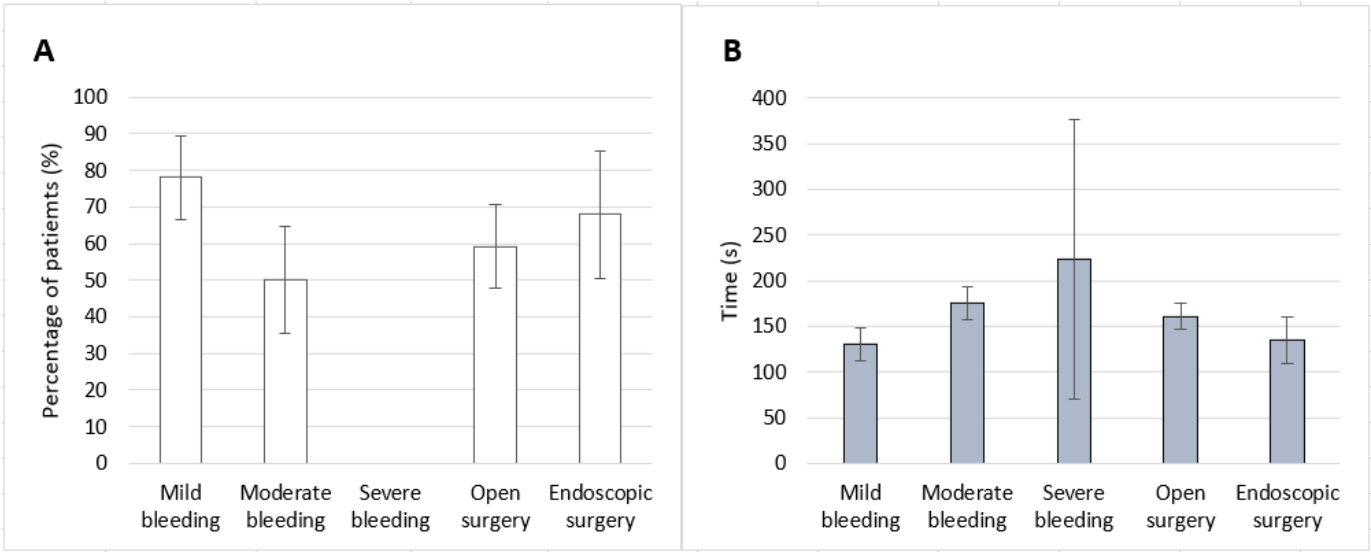

Fig. 2 (A) Primary endpoint success rate as percentage with 95% CI in subgroups according to bleeding severity and type of surgery; (B) Mean time to haemostasis in seconds with 95% CI in subgroups according to bleeding severity and type of surgery; PPS, Per Protocol Set.

OTHER PRIMARY OUTCOME MEASURES

To provide a clearer understanding of the average time required to achieve haemostasis, the graph displays the mean TTH for individual subgroups, along with their 95% confidence intervals (Fig. 2). Across the entire study population, the overall mean TTH was 153.8 seconds (95% CI: 141.5–166.1), with the shortest recorded time being 30 seconds.

The total number of patients who achieved haemostasis within 3 minutes without requiring revision surgery within 12 hours was compared to the total number of patients who achieved haemostasis within 5 minutes without requiring revision surgery within 12 hours.

Regarding the degree of bleeding, patients in minimally invasive procedures, where the endoscopic applicator was used, had predominantly mild bleeding (61.5%). In contrast, moderate bleeding was more common in open surgeries (53.3%).

Tab. 4 Proportion of patients with the same degree of bleeding PPS.

Clinical parameter	Traumacel PULVIS n (%) N = 60	Traumacel PULVIS + Traumacel ENDO Applicator n (%) N = 39	Total n (%) N = 99 (PPS)
Degree of bleeding, n			
1 = mild bleeding	26 (43.3)	24 (61.5)	50 (50.5)
2 = moderate bleeding	32 (53.3)	14 (35.9)	46 (46.5)
3 = severe bleeding	2 (3.4)	1 (2.6)	3 (3.0)
4 = life-threatening bleeding	0	0	0
Degree of bleeding, mean (SD)	1.60 (0.56)	1.41 (0.55)	1.53 (0.56)

SD, standard deviation; TTH, time to haemostasis.

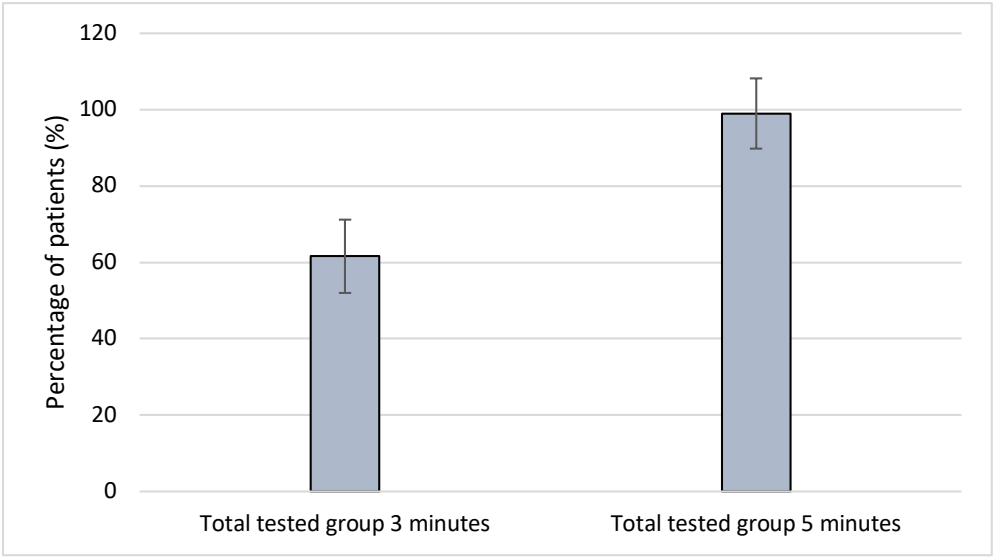

Fig. 3 Proportions of patients in total tested group with bleeding control within 3 and 5 minutes and no need for revision within 12 hours after surgery (with 95% CI); PPS, Per Protocol Set.

This appears to be due to the generally higher tissue bleeding in open surgery than in mini-invasive procedures.

The success rates of 61.6% (95% CI: [52.0, 71.2]) for achieving haemostasis within 3 minutes and 99.0% (95% CI: [97.0, 100.0]) within 5 minutes, without the need for surgical revisions within 12 hours, align closely with data from a similar study (7) on OC fibrillar haemostatic agents. That study reported a success rate of 68.4% (95% CI: [59.2, 77.6]) for achieving haemostasis within 3 minutes and 95.0% (95% CI: [90.5, 99.25]) within 5 minutes, also without revision surgery in 12 hours. Additionally, a comparison of average TTH showed similar results, with this study reporting a mean TTH of 153.8 seconds (95% CI: [141.5, 166.1]) versus 155.94 seconds (95% CI: [141.28, 170.72]) in the fibrillar OC haemostat study. A paired t-test found no statistically significant difference between the mean TTH values ($p = 0.39$). Similarly, when comparing the average degree of bleeding across all PPS patients in this study (1.53) with the average degree of bleeding in the fibrillar haemostat study (1.58), no significant difference was observed ($p = 0.26$). These findings indicate that the degree of bleeding likely does not influence the measured TTH data.

SAFETY OUTCOMES

No device-related adverse events were reported during the study. Postoperative complications were minimal, occurring in 2% of cases, and were unrelated to the haemostatic agent or applicator.

Tab. 5 Safety outcome measures.

Clinical parameter	Traumacel PULVIS n (%) N = 60	Traumacel PULVIS + Traumacel ENDO Applicator n (%) N = 40	Total n (%) N = 100
Complications, n (%)	2 (3.3)	0 (0)	2 (2.0)
AEs, n (%)	0 (0)	0 (0)	0 (0)
AEs at 1-month follow-up, n (%)	0 (0)	0 (0)	0 (0)
Unscheduled visits, n (%)	0 (0)	0 (0)	0 (0)

AEs, adverse events

These postoperative complications occurred: a vein graft ligature failure resulting in rebleeding one-hour post-surgery and bleeding from the chest wall following pleural removal, occurring 13 hours postoperatively. The low number of complications observed aligns with the results of a comparable study involving OC fibrillar haemostats, which reported two complications (2%) unrelated to the haemostatic agent. This consistency supports the strong safety profile of oxidized cellulose-based haemostats, as demonstrated in the efficacy comparisons.

USING THE ENDOSCOPIC APPLICATOR

Endoscopic applicator was used in 40% of cases, predominantly in laparoscopic and thoracoscopic procedures. Its precision delivery of the OC powder contributed to faster haemostasis, particularly in anatomically challenging areas.

The applicator was typically passed through the port and positioned near the target bleeding site (TBS) using a laparoscopic dissector; however, in some instances, the dissector was not required. The data indicate that tools were not necessary for every application of the device, with tools being used in a total of 24 recorded cases.

Tab. 6 Overview of used tools.

Area of surgery	Number of usages of Traumacel ENDO Applicator n (%) N = 40 (SS)	Used Tools N = 24
Thoracic surgery	16 (40.0)	trocar of the brand Storz (6) mediastinoscope of the brand Wolf (3)
Cardiovascular surgery	7 (17.5)	tweezer (1)
General surgery	9 (22.5)	trocar without the specified brand with laparoscopic dissector (8) trocar without the specified brand only (2)
Vascular surgery	0 (0.0)	–
Plastic surgery	0 (0.0)	–
Neurosurgery	3 (7.5)	–
Urology	5 (12.5)	trocar of the brand Storz (3) trocar of the brand Olympus (1) trocar without the specified brand (1)
Department of Anesthesiology, Resuscitation	0 (0)	

DISCUSSION

The findings of this study confirm the efficacy and safety of oxidized cellulose (OC) powder as a versatile haemostatic agent across a variety of surgical scenarios. The use of the endoscopic applicator further enhances the precision and applicability of OC powder, particularly in minimally invasive procedures, where the challenges of accessibility and bleeding control are heightened.

By achieving haemostasis in 61.6% of patients within three minutes and 99.0% within five minutes without revision surgery, the OC powder demonstrates results that are consistent with, and in some cases comparable to, historical data from studies on various OC haemostats.

When comparing the 5-minute success rate of 99% from this study with similar published data, a study comparing knitted non-regenerated OC and regenerated OC haemostats reported a 71.1% success rate for regenerated OC and 89.2% for non-regenerated OC in achieving haemostasis within 5 minutes [11]. Additionally, another

post-market clinical study evaluating a haemostatic powder based on regenerated OC reported a 5-minute haemostatic success rate of 87.5% (9).

A closer look at the subgroup analysis reveals that outcomes vary based on the severity of bleeding and the type of surgery. Patients with mild bleeding demonstrated a higher rate of achieving the primary endpoint (78%) compared to those with moderate bleeding (50%) and severe bleeding (0%), highlighting the importance of bleeding severity as a determining factor for haemostatic efficacy. Although the primary endpoint was not achieved in patients with severe bleeding, suggesting that the OC powder may not be suitable for such cases, haemostasis was nonetheless achieved within 4 minutes in these individuals, and no complications were reported. Common efficacy parameters were chosen, studies on haemostatic agents often compare the proportion of patients achieving haemostasis within a specific time frame (9–11) or analyse the average TTH (11–13). Additionally, other methods are employed, such as calculating blood loss based on haematocrit and haemoglobin levels (14) or measuring the volume of blood collected in surgical drains (15).

Given the absence of device-related AEs, no further statistical tests were done for safety outcomes. The low complication rate aligns with historical safety data for oxidized cellulose-based haemostats (8). However, in rare cases, complications may arise. Several published studies have reported instances of foreign body reactions or hypersensitivity responses (16, 17).

The single-arm design, while practical, limits direct comparisons with alternative haemostatic agents. Future randomized controlled trials could provide more robust evidence. Additionally, exploring the use of these devices in specialized procedures, such as paediatric or robotic surgeries, could expand their applicability.

CONCLUSION

The results of this study highlight the consistent performance of OC powder and its endoscopic applicator across diverse surgical settings and confirm their role as indispensable tools for modern surgical practice. These findings support their continued use across a wide range of surgical disciplines, ensuring effective bleeding control and improved patient outcomes. The absence of device-related adverse events further underscores the safety profile of these products.

CONFLICT OF INTEREST

V. Sívková is an employee of BIOSTER, a. s. Other authors confirm that there are no conflicts of interest associated with this publication.

ABBREVIATIONS

OC	oxidized cellulose
OC powder	the oxidized cellulose hydrogen calcium salt powder
TTH	time to haemostasis
CI	confidence interval
SS	safety set
PPS	per protocol set

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Relationship Between XRCC1 Arg399gln Polymorphism and Risk of Luminal Subtype Breast Cancer in Bali, Indonesia

I Wayan Gede Sutadarma[1,4,*], I Gede Putu Supadmanaba[1,4], Putu Anda Tusta Adiputra[2], Anggi Amanda Triana Devy[3], Anak Agung Bagus Putra Indrakusuma[3], I Gede Aswin Parisya Sasmana[3]

ABSTRACT

Background: Breast cancer is the second leading cause of cancer-related death and the most common type of cancer in women. Recent studies have shown that the development of carcinogenesis is influenced by impaired XRCC1 expression. Therefore, research on the relationship between the XRCC1 Arg399Gln polymorphism and the luminal subtype of breast cancer is important so that it can be used as a reference for further research development.

Methods: This study lasted for 12 months at the Integrated Biomedical Laboratory and Biochemistry Laboratory, Faculty of Medicine, Udayana University. The samples consisted of 30 samples of stored biological material from previous studies with a case-control study design. The status of the XRCC1 Arg399Gln polymorphism was determined by performing PCR on blood samples. Furthermore, the samples were analyzed with SPSS version 25.0.

Results: The number of samples in this study was 15 cases and 15 controls with the majority aged > 50 years. The results of the analysis showed that differences in age groups, menstrual status, and cancer grade were significantly associated with breast cancer subtypes ($p < 0.05$). Based on the results of sequencing and bivariate analysis, the XRCC1 Arg399Gln polymorphism acted as a protective risk factor for the development of luminal subtype breast cancer (OR = 0.182; $p = 0.028$).

Conclusion: XRCC1 Arg399Gln polymorphism is associated with the risk of luminal subtype breast cancer in Bali.

KEYWORDS

breast cancer; polymorphism; luminal subtype; XRCC1 Arg399Gln

AUTHOR AFFILIATIONS

[1] Department of Biochemistry, Faculty of Medicine, Udayana University, Indonesia
[2] Division of Surgical Oncology, Department of Surgery, Faculty of Medicine, Udayana University – Prof. Dr. IGNG Ngoerah General Hospital, Denpasar, Indonesia
[3] Faculty of Medicine, Udayana University, Indonesia
[4] Department of Clinical Nutrition, Faculty of Medicine, Udayana University – Prof. Dr. IGNG Ngoerah General Hospital, Denpasar, Indonesia
* Corresponding author: Department of Biochemistry, Faculty of Medicine, Udayana University, Prof. Dr. IGNG Ngoerah General Hospital, Jl. Diponegoro, No. 45, Denpasar, Bali, Indonesia; sutadarma@yahoo.com

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INTRODUCTION

Breast cancer is one of the most common types of cancer found in women and is responsible for the second leading cause of death in women from cancer. In general, breast tissue consists of two types, namely glandular tissue and supporting tissue (stromal) (1). Breast glandular tissue includes the mammary glands and milk ducts. When neoplasms form, breast cells will grow and settle in the breast tissue as tumors that develop into malignant tumors and undergo metastasis (2, 3). A total of 627 thousand deaths were recorded in 2018 with 1.5 million women diagnosed with breast cancer each year. The incidence of breast cancer in Indonesia reaches 42.1 per 100 thousand population with an average mortality rate of 17 per 100 thousand population. In addition, as many as 80% of breast cancer cases are generally found at an advanced stage so treatment efforts are difficult (4–6).

The etiology of breast cancer is usually multifactorial and can interact with several risk factors such as age, gender, histology, genealogy, reproduction, hormonal, and genetic factors, such as mutations in the BRCA1, BRCA2, and TP53 genes (7). Breast cancer can be classified based on gene expression profiles (subtypes) consisting of Luminal A, Luminal B, and HER2, and basal-like (triple negative) (8). The luminal subtype is a subtype of breast cancer with the highest prevalence and diverse prognosis. Although the aggressiveness of this subtype tends to be lower when compared to TNBC, the high prevalence, and resistance to chemotherapy make research on luminal subtype breast cancer require more attention to be studied. The high prevalence of luminal subtype breast cancer and the many risk factors associated with breast cancer have led to the continued development of breast cancer prognostic markers, one of which is the XRCC1 (X-ray repair cross-complementing 1) gene polymorphism (9).

The XRCC1 gene is a gene that encodes a protein involved in base excision repair (10–12). The protein encoded by XRCC1 functions as a protein that interacts with 8-oxo guanine DNA glycoside, DNA ligase III, DNA polymerase β, and poly (ADP-ribose) polymerase at the site of damaged DNA. One study discussed the role of XRCC1 and showed that the XRCC1 Arg399Gln polymorphism is associated with the development of breast cancer (13). Other studies have also shown a relationship between the XRCC1 Arg399Gln polymorphism and the distribution of breast cancer subtypes. In this study, it was found that the dominant subtype distribution was the luminal subtype. The XRCC1 gene itself is located on chromosome 19q13.2 (13).

Based on this description, in-depth research is needed regarding the relationship between the XRCC1 Arg399Gln polymorphism and the luminal subtype in breast cancer cases, plus the very limited research in Indonesia that examines the relationship between the XRCC1 Arg399Gln gene polymorphism and the risk of luminal subtype breast cancer, especially in Bali. Therefore, research is needed to determine the relationship between the XRCC1 Arg399Gln gene polymorphism and its relationship with luminal subtype breast cancer at Prof. Dr. IGNG Ngoerah General Hospital, Bali, Indonesia.

MATERIALS AND METHODS

STUDY DESIGN AND LOCATION

This study is an analytical study with a case-control study design to see the relationship between the XRCC1 Arg-399Gln polymorphism and the risk of luminal subtype breast cancer in Bali. The study was conducted at the Integrated Biomedical Laboratory and Biochemistry Laboratory, Faculty of Medicine, Udayana University. The study implementation period started from May 2023 to October 2023.

STUDY POPULATION

The accessible population is breast cancer patients who have had venous blood samples taken and stored as Stored Biological Material in the Department of Biochemistry, Faculty of Medicine, Udayana University, Bali, Indonesia. All participants have signed informed consent for the use of samples for research purposes following the Declaration of Helsinki. The sample size is calculated using the following sample size formula (14). The minimum sample size is 15 samples. Thus, the sample size for each group is 15 samples and the total sample size is 30 samples.

RESEARCH PROCEDURE

Subjects were collected using the purposive consecutive sampling method. The samples used in this study were stored biological materials obtained from cancer patients at the oncology polyclinic of Prof. Dr. Soetomo General Hospital IGNG Ngoerah. Basic characteristic data of the research sample were collected from medical record data. Since the accessible population was divided into two groups, the sample selection in this study was also divided into case and control groups. Sample selection with matching criteria according to the research design. Serum from blood samples will be subjected to a polymerase chain reaction to determine the XRCC1 Arg399Gln polymorphism. PCR results are continued with electrophoresis to provide an overview and interpretation of the polymorphism that occurs.

DATA ANALYSIS

The data obtained were then collected. After that, the data was analyzed with SPSS ver 25.

RESULTS

CHARACTERISTICS OF RESEARCH SUBJECTS

Based on the results of this study, it was found that the majority of the age of the research sample was >50 years (14 people; 93.3% for luminal, and 8 people; 53.3% for non-luminal). In the results of the bivariate analysis, the difference in age groups was significantly associated with breast cancer subtypes (p-value = 0.035). When viewed from the menstrual status, most of the research samples were post-menstrual patients as many as 12 people (80%) in the luminal subtype, but in non-luminal it was dominated by

Tab. 1 Characteristics of Research Subjects.

Variable	Luminal (n = 15)	Non-Luminal (n = 15)	OR	CI 95%	p-value
Age					
>50 years old	14 (93.3%)	8 (53.3%)	12.250	1.268–118.362	0.035*
≤50 years old	1 (6.7%)	7 (46.7%)			
Menstrual status					
Post-menopause	12 (80%)	6 (40%)	6.000	1.172–30.725	0.025*
Pre-menopause	3 (20%)	9 (60%)			
Stage					
Early Stage	5 (33.3%)	3 (20%)	2.000	0.381–10.511	0.682
Late Stage	10 (66.7%)	12 (80%)			
Grade					
I–II	14 (93.3%)	8 (53.3%)	12.250	1.268–118.362	0.035*
III	1 (6.7%)	7 (46.7%)			
T Stage					
T1–T2	5 (33.3%)	3 (20%)	2.000	0.381–10.511	0.682
T3–T4	10 (66.7%)	12 (80%)			
N Stage					
N0–N1	5 (33.3%)	3 (20%)	2.000	0.381–10.511	0.682
N2–N3	10 (66.7%)	12 (80%)			
M Stage					
M0	12 (80%)	11 (73.3%)	1.455	0.264–8.009	0.666
M1	3 (20%)	4 (26.7%)			

* The analysis was performed using the Chi-Square Test. The results were considered significant if p ≤ 0.05.

pre-menopausal women as many as 9 people (60%). In the bivariate analysis, a significant relationship was found between subtype and menstrual status (p-value = 0.025). Based on the stage, most patients were diagnosed at the final stage as many as 10 people (66.7%) in the luminal subtype, and 12 people (80%) in the non-luminal subtype. This proportion is then similar to the variables of tumor size and lymph node metastasis, although no statistically significant relationship was found in bivariate analysis. When evaluated on cancer grade, most of the study samples were found to have grade I–II, wherein the luminal group there were 14 people (93.3%), and in the non-luminal group, there were 8 people (53.3%), whereas in bivariate analysis this result was statistically significant with p-value = 0.035 (Tab. 1).

XRCC1 ARG399GLN POLYMORPHISM IN BREAST CANCER PATIENTS

Based on the results of this study which aims to evaluate the relationship between XRCC1 ARG399GLN gene polymorphism in breast cancer patients, it was found that there was a dominant proportion related to polymorphisms that occurred in the non-luminal subtype of 11 people (73.3%). On the other hand, only a small portion of the study samples had polymorphisms in the luminal subtype, namely 5 people (33.3%) of the total number of samples in the luminal subtype group (Tab. 2).

Tab. 2 Proportion of XRCC1 ARG399GLN Gene Polymorphism Occurrence in Breast Cancer Patients.

Variable	Luminal (n = 15)	Non-Luminal (n = 15)
Polymorphism status		
Polymorphism	5 (33,3%)	11 (73.3%)
Non-Polymorphism	10 (66.7%)	4 (26.7%)

RELATIONSHIP OF XRCC1 ARG399GLN POLYMORPHISM WITH LUMINAL SUBTYPE BREAST CANCER RISK

When reviewed regarding the relationship of XRCC1 ARG399GLN gene polymorphism with the risk of luminal subtype breast cancer, this study found that XRCC1 ARG399GLN gene polymorphism acts as a protective risk factor for the development of luminal subtype breast cancer with an OR value of 0.182; 95% CI 0.038–0.873 (Tab. 3).

Tab. 3 Relationship of XRCC1 ARG399GLN Polymorphism with Luminal Subtype Breast Cancer Risk.

Variable	Polymorphism status		OR	CI 95%	p-value
	Polymorphism	Non-Polymorphism			
Luminal	5 (33.3%)	10 (66.7%)	0.182	0.038–0.873	0.028*
Non-Luminal	11 (73.3%)	4 (26.7%)			

DISCUSSION

Base excision repair (BER) is a process of repairing localized DNA damage due to oxidative stress and ionizing radiation. The XRCC1 gene (X-ray repair cross-complementing group 1) is one of the genes involved in the DNA base excision repair process and maintaining genetic stability. XRCC1 has three different domains that interact with poly (ADP-ribose) polymerase, DNA polymerase b, and DNA ligase III. Recent studies have shown that XRCC1 polymorphisms affect the risk of breast cancer. Commonly studied polymorphisms are Arg194Trp, Arg399Gln, and Arg280His. Women will have a high risk of breast cancer when there are XRCC1 gene polymorphisms, including Arg399Gln which have been reported by various previous studies with some similarities in demographic characteristics in this study (15, 16).

Women aged >50 years and postmenopausal tend to have luminal breast cancer in this study. In addition, the luminal subtype was found more in grades I–II. These results follow the study by Mills et al (2020) that women with ER+/HER2– breast cancer aged ≥75 years have a more aggressive incidence of luminal type B cancer. It was also found that the luminal A and B subtypes were found most in grade II, with tumor size >2 cm for luminal B, and no lymph node metastasis in luminal A, but there was in luminal B (17). However, Zhang et al (2019) found that postmenopausal women experienced HER2 and basal-like subtypes more often than luminal subtypes. Other studies have suggested that delayed menopause is associated with an increased risk of basal and luminal tumors (18).

A study by Akhzari et al (2018) showed that XRCC1 gene polymorphism in 150 breast cancer patients had 76.67% heterozygosity and 27.87% homozygosity. Meanwhile, the probability of the patient group with heterozygous genotype (Arg/Gln) was higher compared to homozygosity (19). Luminal breast cancer is clinically low-stage, slow-growing, and has a good prognosis with a lower incidence of recurrence and a higher survival rate. This carcinoma has a high response rate to hormone therapy (tamoxifen or aromatase inhibitors), and the benefits of chemotherapy are more limited. Luminal B breast cancer has a higher grade and worse prognosis compared to luminal A. This tumor is ER positive and PR negative and has high Ki67 expression (more than 20%) (20).

XRCC1 polymorphisms were significantly associated with breast cancer subtypes ($p<0.05$). XRCC1 polymorphisms showed high frequencies in luminal A, HER2-positive, and TNBC breast cancers (21). Meta-analysis showed a significant association between XRCC1 Arg399Gln polymorphism and breast cancer risk (OR for dominant model = 1.12, 95%CI: 1.02–1.24, $P_{herogeneity}$ = 0.003; OR for additive model = 1.07, 95%CI: 1.01–1.14, $P_{heterogeneity}$ = 0.017) (22). Non-luminal breast cancer subtypes such as TNBC are highly aggressive phenotypes associated with poor prognosis. This condition is characterized by a lack of expression of estrogen receptors (ER), progesterone receptors (PR), and epidermal growth factor receptor-2 (HER2) (23, 24). The absence of these markers leads to rapid metastasis, treatment resistance, and high recurrence rates. Breast cancer cells with higher-than-normal HER2 levels are called HER2-positive. These cancers tend to grow and spread faster than HER2-negative breast cancers but are more likely to respond to treatment with drugs that target the HER2 protein (25).

CONCLUSION

Based on the results of the study on the analysis of the relationship between the XRCC1 Arg399Gln polymorphism and the risk of luminal subtype breast cancer in the oncology polyclinic of Prof. Dr. IGNG Ngoerah Hospital, Denpasar, Bali, it was found that individuals who have the XRCC1 Arg399Gln polymorphism have a lower risk of developing luminal subtype breast cancer compared to non-luminal subtypes. Therefore, it can be concluded that the XRCC1 Arg399Gln polymorphism has a protective effect on the development of luminal subtype breast cancer.

CONFLICT OF INTEREST

None.

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Intra-Operative and Post-Operative Impact of Body Weight on Total Hip Arthroplasty: Are We Approaching It Correctly?

Edoardo Ipponi[1,*], Sara Barderi[1], Elena Bechini[1], Belinda Misso[1], Luca Orsetti[1], Lorenzo Andreani[1], Nicola Piolanti[1], Enrico Bonicoli[1], Paolo Domenico Parchi[1]

ABSTRACT

Background: To this date, the impact of body weight on total hip arthroplasty is still debated. The literature lacks evidence on the impact of body weight on surgical times and complications.

Methods: We retrospectively evaluated all the patients who underwent primary total hip arthroplasty (THA), collecting patients' pre-operative BMI before surgery. We recorded the surgical approach performed (anterior or posterolateral), and their duration. All the major complications were recorded.

Results: Seven-hundred-thirty-two cases were included (627 posterolateral and 105 anterior approach). The mean BMI was 27.3. The mean surgical time was 90.8 minutes (104.9 for anterior and 88.5 for posterolateral approach). We found a significant positive relationship between BMI and surgical times for both cases treated with a posterolateral approach, and (even more remarked) in case anterior approach. Thirty-nine cases (5.3%) had major complications; 27 of them (69.2%) were overweight or obese. The surgical times of those who had complications were significantly higher compared to others.

Conclusions: Patients' body weight, and in particular their Body Mass index, has a direct impact on the duration of THA surgical procedures, particularly if performed using an anterior approach, and an indirect effect on complication rates.

KEYWORDS

BMI; THA; arthroplasty; osteoarthritis; anterior approach; posterolateral approach

AUTHOR AFFILIATIONS

[1] University of Pisa – Department of Orthopedics and Trauma Surgery, Pisa, Italy

* Corresponding author: University of Pisa – Department of Orthopedics and Trauma Surgery, Via Paradisa 2, 56124 Pisa, Italy; edward.ippo@gmail.com

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INTRODUCTION

Obesity is a pathology on the rise, as it has reached epidemic proportions in many Western countries (1, 2). Obesity is associated with a series of other pathologies, such as asthma, type 2 diabetes, hypertension, sleep apnea, and cardiovascular diseases due to weight gain and the accumulation of visceral adiposity, which increases the release of pro-inflammatory hormones and cytokines (3–5). All these comorbidities concur to make obesity a serious issue for individuals' health and a major source of cost for national healthcare systems all over the world (1, 2).

Increased adiposity and a higher body mass index (BMI) also impact the musculoskeletal system by increasing the joint load and progressively leading to osteoarthritis. Characterized by loss of articular cartilage, bone hypertrophy, and joint capsule thickening, osteoarthritis is a progressive pathology that causes joint pain and consequent functional limitation, particularly in the hip joint (5, 6).

Although conservative approaches like the administration of NSAIDs or local infiltrations can temporarily counteract the clinical symptoms and delay articular degeneration, surgery with prosthetic replacement still represents the treatment of choice for advanced hip osteoarthritis (7–9).

Body mass and weight enhance articular degeneration, increase the rates of earlier prosthetic replacements, and, according to some authors, may also be responsible for complications, including infections and wound dehiscences (10, 11).

This study aims to evaluate the correlation between patients' BMI, operating times, and peri- and postoperative complications between two groups of patients treated with total hip arthroplasty (THA) using anterior and posterolateral approach.

MATERIALS AND METHODS

This single-center retrospective study was conducted according to the ethical standards in the 1964 Declaration of Helsinki and its later amendments.

Our study consisted of a review of all patients with hip osteoarthritis treated with THA in our institution between January 2020 and December 2023. The data of all patients were collected in a digital database. Inclusion criteria were (I) a pre-operative diagnosis of primary hip osteoarthritis (grade II–III according to the Tönnis classification), (II) an anterior or posterolateral surgical approach, (III) a primary implant with cementless prosthesis, and (IV) a follow-up longer than 12 months. Exclusion criteria were (I) a hip fracture at the moment of hospitalization, (II) a history of avascular necrosis of the femur head, (II) previous surgeries in the treated hip of the nearby inguinal or gluteal region, and (IV) pre-operative neurological deficits or other systemic diseases that could have impeded a proper rehabilitation, and reduced patients' mobilization after surgery. All surgical procedures were performed by high-volume surgeons, experts in both surgical approaches. The approach of choice was chosen by the single surgeons based on their personal preference for the anatomical characteristics of each single patient.

Patients were divided into two groups depending on the surgical approach performed. Group A included cases treated with a posterolateral approach, whereas patients that received an anterior approach to their hip were included in Group B.

We collected general data for each patient, including age, gender, height, and weight at the moment of surgery. The latter were used to calculate the BMI. We recorded the duration of each procedure for each patient. We reported each intraoperative or postoperative complication with a Clavien-Dindo grade III or higher.

Follow-up data were collected and recorded in our database as patients underwent our standardized postoperative protocol. The protocol consisted of serial office visits, clinical evaluations, and postoperative X-rays performed within one month (clinical evaluation only) and later 3, 6, and 12 months after surgery. Further evaluations were scheduled on a yearly basis, with eventual changes depending on each patient's needs.

STATISTICS

Statistical analysis was performed using Stata SE 13 (StataCorp LLC, College Station, TX). Statistical significance was set at 0.05 for all endpoints.

RESULTS

Seven hundred thirty-two cases met our inclusion criteria and were thereby included in our study. There were 402 females and 330 males, with a mean age of 74.7 years (48–87). Six hundred twenty-seven cases had a posterolateral approach and were therefore included in Group A, whereas 105 cases received an anterior approach and were enrolled in Group B. The mean BMI of our whole population was 27.2 (16.9–48.7): 27.4 (17.5–48.7) for Group A and 25.8 (16.9–39.3) for Group B. Among our 732 patients, 311 (43.5%) were overweight and 172 (23.5%) were obese. In particular, within Group A, 206 (33%) were normal weight or underweight, 262 (42%) were overweight, and 157 were obese (25%). Group B had 45 normal weight or underweight cases (43%), as many overweight, and 15 obese (14%).

The mean duration of surgical procedures was 90.8 (30–235) minutes: 88.5 (30–235) for posterolateral approaches (Group A) and 104 (50–180) for anterior approaches (Group B). A Pearson correlation test reported a statistically significant direct correlation between all patients' BMI and surgical times ($r = 0.148$; $p = 0.00005$). Performing the same test separately for the two groups, although the correlation was statistically significant also for Group A ($r = 0.113$; $p = 0.00443$), we observed a particularly strong correlation between BMI and surgical times in Group B ($r = 0.478$; $p < 0.00001$).

Thirty-seven cases, accounting for 5.0% of all treated patients, had significant complications. The most frequent complications were infections (12), aseptic loosening (7), periprosthetic fractures (5), hematomas, and neurological damage (3). Table 1 details all complication types.

Tab. 1 A schematic resume of our population, sorted in the middle into two groups according to the surgical approach and on the left into three columns according to the patient's BMI.

	TOTAL	GROUP A (Posterolateral)	GROUP B (Anterior)	Normal Weight (BMI < 25)	Over Weight (25 < BMI < 30)	Obese (BMI > 30)
Number of cases	**732**	**627**	**105**	**249**	**311**	**172**
Mean BMI	**27.2** (16.9–48.7)	**27.4** (17.5–48.7)	**25.8** (16.9–39.3)			
Mean surgical time	**90.8** (30–235)	**88.5** (30–235)	**104.9** (50–180)	**88.5** (30–200)	**90.1** (35–235)	**95.3** (55–180)
Complications	**37**	**31** (84%*)	**6** (16%*)	9 (24%*)	18 (49%*)	**10** (27%*)
Infections	12	11	1	1	9	2
Aseptic loosening	7	7	0	1	3	3
Periprosthetic fracture	5	3	2	1	3	1
Neurological damage	3	3	0	1	1	1
Hematoma	3	2	1	1	1	1
Seroma	2	2	0	2	0	0
Wound dehiscence	2	2	0	1	0	1
Other	3	1	2	1	1	1

* Relative percentage among all complications.

Twenty-eight of the 37 patients who had complications (76%) were overweight (18; 49%) or obese (10; 27%). The mean BMI of those who developed complications was 28.8 (22.0–43.0). A Withney-Mann test reported that those who developed complications had a significantly higher BMI compared to those who did not develop any complication ($p = 0.02341$). Furthermore, according to another Withney-Mann test ($p = 0.00059$), the surgical times of those who had complications were significantly higher than those who did not develop complications. Our cohort's size was insufficient to detect whether one surgical approach had a higher complication rate than another.

DISCUSSION

Surgical timing is known to have a crucial role in determining the outcomes of hip arthroplasty (12, 13). Limiting surgical times intuitively shortens the duration of anesthesia, reduces blood loss, and minimizes the risk of infection by restraining the exposition of the surgical site (14–16).

That increase in operating time can be attributed to several aspects.

Intuitively, tissue dissection is required to reach the joint plane, and larger adipose masses require longer dissections. At the end of the procedure, the same goes for suturing the access, a step that takes more time in patients with wide subcutis surfaces.

These latter may not allow for tracing the landmarks necessary to perform the surgical approach and expose the acetabulum, and may limit exposure of the joint unless largely extending the skin incision (17).

Furthermore, the intraoperative moves to place and reduce the joint prosthesis can be more difficult due to the increase in the masses to maneuver (18).

Finally, as suggested by Cannata et al. (19), the link between body weight and surgical time could also be related to the comorbidities that, in many cases, accompany obese patients.

Our study demonstrates a statistically significant linear correlation between patients' BMI and surgical times. On average, heavier patients had longer surgeries. Although this correlation was significant for both anterior and posterolateral approaches, it was noticed that the correlation between high BMI and increased surgical time was particularly evident in anterior hip arthroplasty operations.

Some authors already investigated the influence of BMI on anterior THA surgical timing, mostly focusing on obese patients (19, 20). Cannata et al. (19) and Antoniadis et al. (20) and showed increased operating times in THA surgery with direct anterior access. Russo et al. (21) found greater success and fewer complications in normal-weight patients than patients with high BMI during the direct anterior approach. Our findings are consistent with those of Russo et al. and allow for a direct comparison between two of the most used surgical approaches to the hip. According to our experience, the anterior approach seemed to be the most sensitive to BMI variations, and this difference should be considered by surgeons who are experts in both techniques and face severely overweight and obese cases.

Several studies in modern literature suggest that increased body mass index, overweight, and particularly obesity also increase the risk of complications directly or indirectly related to orthopedic surgical procedures (19, 22–26).

In a recent paper, Schmerler et al. (27) analyzed the outcomes of a survey on over eighty thousand patients who underwent revision total hip arthroplasty in the United States of America. Their results suggest that BMI was associated with the rates of some of the most common com-

plications in hip arthroplasty. Compared to normal-weight patients, overweight and obese patients were 10% more likely to have a revision due to periprosthetic joint infections (PJIs). However, the same study also reported that obese patients were 19% less likely to have a revision due to dislocation, suggesting that the patient's weight and BMI might modulate the relative risk of complications rather than increasing their overall likelihood.

The impact of obesity on postoperative complications was also evaluated on an even larger scale by Onggo et al. (23), who published a literature review on over two million THA patients from sixty-seven studies. According to their findings, obese patients had higher global complication rates and were significantly more at risk of developing infections and dislocations compared to non-obese people.

Our outcomes mainly align with these observations. In our population, those who developed complications had a significantly higher BMI compared to those who did not develop any complications. Our cumulative complication rate was 3.6% for normal-weight patients, whereas it rose to 5.8% for both overweight (BMI between 25 and 30) and obese patients (BMI higher than 30). These data suggest that not only obesity but also overweight could be exposed to a higher risk of complications after THA.

Infections and dislocations are among the most common complications in THA surgery (28, 29). The lack of revision surgeries due to dislocations did not allow us to evaluate the impact of BMI on this eventuality. Infections, instead, represented the most frequent reason for prosthetic failure in our cohort and were more common among overweight and obese patients than normal-weight patients.

In our study, surgical times and the risk of developing postoperative complications were directly correlated, as the surgical times of those with complications were significantly higher than those of the remaining patients. In our population, surgical time was also linked with BMI, therefore increments of this latter could indirectly raise the risk of postoperative complications.

We are aware that our study is not free of limitations.

The retrospective nature of our study did not allow the complete standardization of intraoperative procedures and postoperative follow-ups for each patient. Another limitation is the monocentric nature of our study, which limited the number of available cases and partially limited the statistical significance of some of the data associations we wanted to investigate at the beginning of our research. These limits could be overcome by performing similar evaluations on a prospective basis and with broader populations.

Beyond these limits, our findings could increase our knowledge of BMI's impact on THA surgery. In our study, an increased BMI led to significantly longer surgical times in patients treated using both posterolateral and anterior approach, with this latter being the most sensitive to body mass variations. Furthermore, patients with a higher BMI were most likely to develop postoperative complications and require revision surgeries. These findings confirm the importance of weight in determining the ease of the surgical procedure alone and the success of the whole treatment.

Patients' weight should be considered when planning the surgical approach. They should be made aware that being overweight and obese could increase their risk of developing surgery-related complications and potentially undermine the success of THA. For these reasons, when feasible, overweight patients should be encouraged to lose their weight before surgery and avoid returning to a higher body mass index in the postoperative phase.

CONCLUSION

Patients' body weight, and in particular their Body Mass index, has a direct impact on the duration of THA surgical procedures, particularly if performed using an anterior approach, and an indirect effect on complication rates.

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Two Hurt More Than One: Severe Hyponatraemia and Rhabdomyolysis as Presenting Features of Addison's Disease

Luca Foppiani[1,*], Christian Cascio[1], Paola Pesce[2], Giancarlo Antonucci[1]

ABSTRACT

Addison's disease (AD) is characterized by non-specific symptoms and electrolyte disorders, namely hyponatraemia and hyperkalaemia; rhabdomyolysis is uncommon. AD may manifest at onset with a life-threatening adrenal crisis which is triggered by stressful events. We describe the case of a young man who was hospitalized for severe myalgia and fatigue. Severe hypotonic hyponatraemia, rhabdomyolysis and hypotension were found; hormonal assessment unexpectedly revealed primary adrenal insufficiency. Saline infusion and intravenous hydrocortisone significantly improved the patient's condition and normalized sodium and muscle enzyme levels; thereafter, he was switched to oral steroid therapy. The autoimmune origin of AD was ascertained by the positivity of adrenal cortex autoantibodies and 21b-hydroxylase autoantibodies. The association of hyponatraemia and rhabdomyolysis may be the initial finding of an as yet unknown AD, which requires proper investigation and treatment.

KEYWORDS

Addison's disease; rhabdomyolysis; hyponatraemia; myalgia; autoimmune adrenalitis

AUTHOR AFFILIATIONS

[1] Internal Medicine, Galliera Hospital, Genoa, Italy
[2] Autoimmunity Laboratory, San Martino Hospital, Genoa, Italy
* Corresponding author: Internal Medicine, Galliera Hospital, Mura delle Cappuccine 14, 16128 Genova, Italy; luca.foppiani@galliera.it

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INTRODUCTION

AD is an uncommon (incidence of 4–6 cases/million/year) and frequently overlooked disease (1–3); the autoimmune form is predominant. AD is manifested through the gradual onset of general symptoms present in many other conditions, so that its diagnosis is often delayed and formulated only in the presence of an adrenal crisis. Electrolyte disturbances such as hyponatraemia and hyperkalaemia (1–4) are characteristic of this disease. Rhabdomyolysis is an uncommon finding in AD (5–13), but is often overlooked, since muscle enzyme evaluation is not routinely performed.

We report the case of a young man suffering from worsening muscle aches and fatigue with biochemical findings of severe hyponatraemia and rhabdomyolysis, which led to an unexpected diagnosis of primary adrenal insufficiency of autoimmune etiology.

CASE REPORT

A 26-year-old man was taken to the Emergency Department (ED) of our hospital owing to worsening myalgia involving the lower limbs and fatigue over the previous three months. At that time, he had been evaluated in the ED of another region, owing to nausea and vomiting; severely reduced serum sodium levels (122 mEq/L), potassium levels in the upper normal range and hypotension (85/50 mm Hg) were found. On that occasion, the infusion of saline supplemented with sodium chloride raised sodium levels (129 mEq/L). The patient was discharged with the diagnosis of acute gastroenteritis.

On admission to our ED, the patient was alert, apyretic, and dehydrated. He reported that he was not taking any medication. Blood pressure (BP) was low (90/50 mm Hg), whereas oxygen saturation (98%), heart rate (60 bpm) and lung and abdominal examination were normal. Electrocardiogram and chest X-ray were unremarkable. Venous haemogas analysis showed partially compensated metabolic acidosis with normal anion gap: pH: 7.34, pCO_2: 33.4, HCO_3^-: 19, hyponatraemia: 113 (n. v. 135–145) and hypochloraemia: 83 (n. v. 98–106).

Blood chemistry confirmed the severe hyponatraemia (113 mmol/l) and revealed rhabdomyolysis characterized by increased serum creatine phosphokinase (CPK) and CPKMB levels (2% of total CPK) with normal troponin T levels, and lymphocytosis; creatinine was normal, and potassium was in the high-normal range (Table 1). Following the infusion of 500 ml of saline, the patient was transferred to our internal medicine ward in the late afternoon.

On physical examination, the patient was seen to be thin (BMI: 19 kg/m^2); hypotension was confirmed (90/60 mm Hg), with a further reduction (70/50 mm Hg) during orthostatism. He reported a weight loss of 5 kg during the last few months.

A diagnostic work-up for hyponatraemia was immediately carried out, including evaluation of adrenal gland and thyroid function, uric acid levels, spot urinary sodium levels and urine osmolarity. The urine data, ready available, were 34 mmol/l and 360 mOsm/kg, respectively.

In addition, blood sample was collected to uncover an autoimmune or infectious (cytomegalovirus, Epstein Barr virus, hepatitis B and hepatitis C, immunodeficiency virus) aetiology of rhabdomyolysis.

Afterwards, 500 ml of saline supplemented with 40 mEq of sodium chloride followed by 500 ml of normal saline were infused.

The morning after admission, a closer patient's assessment highlighted hyperpigmentation of both the outer border of the tongue, nail beds and elbow skin.

New blood chemistry confirmed profound hypotonic (serum osmolarity: 233 mOsm/kg) hyponatraemia (116 mmol/l); rhabdomyolysis had significantly worsened in comparison with the admission values, myoglobin levels were four times the upper normal value, uric acid level was in the low-normal range, and potassium levels were in the upper normal range (Table 1). Thyroid function showed a mild increase in TSH levels and normal FT4 levels (Table 1). Autoimmune and infectious tests for rhabdomyolysis were negative.

Remarkably, serum cortisol levels proved nearly undetectable (0.8 μg/dl) and were associated to hugely increased ACTH levels (1500 pg/ml); further rapid evaluation showed markedly increased supine renin levels, aldosterone levels in the low-normal range and severely decreased DHEAS levels (Table 1). The pituitary-gonadal axis was normal (Table 1), and thyroid autoantibodies were negative. Since hormonal results indicated primary adrenal insufficiency and the patient was hypotensive, he was immediately treated with 100 mg intravenous (i. v.) hydrocortisone, which was subsequently administered every 8 hours for two days and then progressively tapered to 50 mg two times a day, together with continuous normal saline infusion (1500 ml/day); accordingly the patient's condition and BP significantly improved.

Before starting hydrocortisone, blood sample was collected for evaluation of adrenal cortex autoantibodies (ACA) and 21b-hydroxylase autoantibodies (21-OH Ab). After a few days of i.v. hydrocortisone therapy, the patient was switched to oral therapy with cortisone acetate (25 mg at 8 a.m., 12.5 mg at 1.00 p.m. and 12.5 mg at 6 p.m.) and 0.05 mg fludrocortisone at 8 a.m. Five days after the beginning of i.v. steroid therapy, sodium, CPK, lymphocyte count and TSH levels normalized, and potassium levels settled in the middle of the normal range.

Both ACA: 1:20 by means of indirect immunofluorescence (Figure 1) and 21-OH Ab: 12 U/ml (n. v. < 0.4) proved positive; accordingly an autoimmune aetiology of the adrenal insufficiency was proved. Autoimmune polyendocrinopathies type 1 and type 2 were excluded by hormonal (Table 1) and physical evaluation. Anti-gastric parietal cell antibodies and anti-transglutaminase antibodies were absent. The patient was eventually discharged in good condition, and the dose of cortisone acetate was reduced to 43.5 mg per day; he was instructed on how to increase glucocorticoid therapy during stressful events, or to switch to parenteral hydrocortisone when required.

Hormonal and biochemical data of patient throughout hospitalization and follow-up are shown in Table 1.

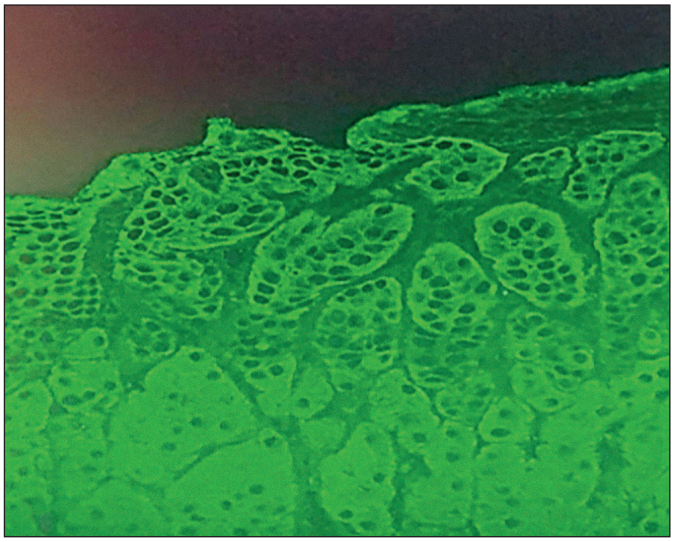

Fig. 1 Indirect immunofluorescence (1:20 dilution of patient's serum) on human unfixed adrenal tissue showing adrenal cortex autoantibodies (ACA) positivity in the adrenal glomerular zone.

APPENDIX

One month after discharge, the patient was hospitalized in another region owing to fever and diarrhoea. Blood chemistry showed neutrophilic leucocytosis (12400/mm^3 [n. v. 4000–9300]), increased C reactive protein and severe hypokalaemia (Table 1). Stool culture was positive for Salmonella, and a diagnosis of gastroenteritis was made. Saline, 100 mg i.v. hydrocortisone every 8 hours, i.v. potassium and 2 g ceftriaxone were started, eliciting rapid improvement of the patient's clinical condition and normalization of potassium levels. Hydrocortisone was tapered to 50 mg i.v. three times a day, and eventually oral steroid therapy was restarted. The patient was subsequently discharged and scheduled for follow-up where he is well and has normal BP and electrolyte levels (Table 1); the dose of cortisone acetate was reduced to 37.5 mg per day.

Tab. 1 Hormonal and biochemical evaluation of patient throughout hospitalization and follow-up.

	DAY 0 Our emergency department admission	DAY 1 start of steroid therapy	DAY 2	DAY 3	DAY 4	DAY 7 discharge	DAY 17 follow-up	DAY 37 Another hospital admission	DAY 57 Follow-up	DAY 112 Follow-up
glycemia (mg/dL)	122 (not fasting)	81	68	83		79	81	72	77	
Hb (14.2–17 gr/dL)	15.4	14.9	14.2	12.3		11.6	12.6	12.9	15.1	15.2
lymphocyte count (10^9/L) (1.13–3.37)	5010	4000	3170	2560		4240	3060		2400	2790
C-reactive protein (0–0.5 mg/dL)	0.1	0.3		0.1				11		
Na (135–145 mEq/L)	113	116	119	124	131	138	137	143	141	141
K (3.5–5 mEq/L)	4.6	4.9	4.9	3.8	3.7	3.8	3.9	2.7	4.1	3.9
creatinine (0.7–1.2)	0.9	0.7	0.7	0.7	0.6	0.8		0.8		
uric acid (3.5–7 mg/dL)	3.5									
calcium (8.2–10.2 mg/dL)	9.7	9.2	8.8			8.9				
CPK (39–308 UI/L)	1042	6652	3897		317	37	52			
myoglobin (28–72 ng/ml)	285									
ACTH (4.5–48 pg/mL)		1500					361.4		74.2	112
cortisol (6.2–19.4 µg/dL)		0.8								
DHEAS (160–449 µg/dL)		26.8								
supine renin (2.4–29 µU/mL)		500					68			75.7
supine aldosterone (3–15 ng/dL)		3.4								
FT4 (0.93–1.7 ng/dL)		1.68					1.56			
TSH (0.27–4.2 µU/mL)		6.77			1.7		3.1			3.0
PTH (15–50 pg/mL)			14.7		19.1				37.3	
calcium (8.5–10.5 mg/dL)	9.7	9.2	9.2		8.8	8.9			10	
25-OHD (> 30 ng/mL)			15.1							
LH (1.7–8.6 mUI/mL)			6.1				3.9			
FSH (1.5–12.4 mUI/mL)			0.7				2.8			
PRL (4–18.4 ng/mL)			32.7				12.5			
testosterone (3–8 ng/mL)			5.7				6.7			8.0
SHBG (10–80 nmol/L)							60.5			

DISCUSSION

The symptoms of AD arise slowly and insidiously; they include fatigue, anorexia, nausea, weight loss and, more rarely, muscle aches (1–4). Under stressful circumstances that require increased cortisol output (infections, surgery, trauma), an addisonian crisis may occur and be life-threatening if not promptly recognized and properly treated (1–4).

The most common electrolyte disorders found in AD are hyponatraemia and hyperkalaemia (1–4) (Table 2). By contrast, rhabdomyolysis is rarely observed and often overlooked (5–13) (Table 2). Our patient attended ED owing to worsening myalgia, which had started three months earlier. At that time, he had been examined at the ED of another region because of vomiting and malaise, and was found to have severe hyponatraemia, which was treated with sodium chloride-supplemented saline. A diagnosis of acute gastroenteritis was made and no further investigations were carried out.

Evaluation at the ED of our hospital confirmed severe hyponatraemia together with rhabdomyolysis; in addition, hypotension was found.

The work-up for hyponatraemia showed reduced serum osmolarity and spot urine sodium levels suggestive of renal loss. Combining anamnestic reports, and biochemical and clinical findings indicated that this was a case of chronic hypotonic hypovolemic hyponatraemia.

In addition, the presence of recent weight loss and hyperpigmentation in typical areas of the body was highlighted; accordingly, the suspicion of primary adrenal insufficiency was put forward, and was confirmed by means of hormonal evaluation. The autoimmune aetiology of AD was ascertained by the the positivity of ACA and 21-OH Ab.

Up to 85% of patients with AD have (hypovolemic) hyponatraemia (4), and sodium levels may be as low as 88 mmol/l (3).

Hyponatraemia is related to two pathophysiological mechanisms: i) the major cause is the reduced/absent action of aldosterone on distal tubules and collecting ducts which causes renal sodium wasting and hypovolaemia. In this regard, spot urine sodium levels in our patient were compatible with activated natriuresis; ii) the additional cause is the increased ADH release secondary to both cortisol deficiency and hypovolemic state (14).

It is well known that cortisol is an inhibitor of ADH secretion; accordingly, hyponatraemia in AD is enhanced by retention of free water related to the inappropriate secretion of ADH (15). The ADH release may also be stimulated by the reduction of blood pressure and cardiac output signalled to the central nervous system by baroceptors located in the carotid sinus and in the aortic arch. Finally, increased renal sensitivity to ADH might be involved, as suggested by aquaporin-2 water channel up-regulation in glucocorticoid-deficient rats (14). On the hand, the euvolemic hyponatremia often ascertained in secondary adrenal insufficiency is basically related to ADH release secondary to cortisol deficiency.

In our patient, sodium levels slightly increased the day after sodium chloride-supplemented saline infusion was started, and progressively normalized with i.v. hydrocortisone and normal saline and subsequent oral steroid therapy.

In the absence of traumatic events or the use of myotoxic drugs and despite the presence of hyponatremia, the cause of the rhabdomyolysis in our patient was initially not so clear. Autoimmune and infective aetiologies were excluded. The slight increase in TSH levels ascertained was not deemed a relevant cause; TSH levels normalized spontaneously a few days after starting steroid therapy.

Rhabdomyolysis is characterized by muscle weakness and/or myalgia, with release into the bloodstream of myofibril enzymes such as CPK and myoglobin (16). It may range from asymptomatic to severe, including compartment syndrome or renal failure due to myoglobin cast formation with subsequent intratubular obstruction. In our patient, the rhabdomyolysis manifested with myalgia and muscle weakness and did not cause renal failure.

The association between rhabdomyolysis and adrenal insufficiency (primary or secondary) is uncommon and overlooked (17–26) (Table 2).

To our knowledge, rhabdomyolysis with adrenal failure without hyponatraemia has been observed in only four cases: in three cases, adrenal insufficiency was of secondary origin, and due to long-term glucocorticoid therapy (one case) (18) and to panhypopituitarism (2 cases) (21, 24), respectively; in the remaining case, it was of primary origin due to an autoimmune process (8). In order to explain rhabdomyolysis in secondary adrenal insufficiency, in which aldosterone function is preserved and sodium levels are often normal, two pathogenetic mechanisms have been put forward: i) impaired muscle perfusion due to relative hypotension (24); ii) altered glycogenolysis and/or impaired mitochondrial oxidative metabolism of the muscle cells (24).

The hyponatraemia-associated rhabdomyolysis has been related to two mechanisms i) the reduced osmolality of the extracellular fluid causes cell swelling and intracellular potassium release into the extracellular space, with a decrease in myocyte transmembrane potential and subsequent muscle breakdown and the release of CPK and myoglobin into the bloodstream; ii) abnormalities in the Na^+/Ca^{++} exchange pump in the cell membrane; as the sodium level in the extracellular fluid decreases, the Ca^{++} output, which is related to the Na^+ input, also falls. Accordingly, intracellular calcium build-up activates proteases and phospholipases that cause myolysis. The rate of decline in serum sodium concentration and the severity of hyponatremia are significantly associated with the severity of muscle injury (16).

In our patient, rhabdomyolysis initially worsened despite the slight increase in sodium levels following the infusion of sodium chloride-supplemented saline. Thereafter, with the initiation of i.v. hydrocortisone for AD and the continuation of normal saline infusion, muscle enzyme levels normalized in a few days and myalgia improved. Rhabdomyolysis was therefore deemed to be caused by the combination of both severe hyponatraemia and hypocortisolism.

A final consideration emerges from the patient's previous medical reports. The diagnosis of AD was delayed by

Tab. 2 Literature summary of clinical and hormonal data and sodium levels in patients with rhabdomyolysis and adrenal insufficiency.

Authors	Age/gender	CPK peak (IU/L)	Sodium (mEq/L)	ACTH (pg/ml)	Cortisol (mg/dl)	Adrenal insufficiency	Comorbid endocrinopathies
Mor et al.	44/F	1670	103	129.4	0.7	primary	nil
Egan et al.	63/F	21490	97	405	18.1 → 6.1	primary	nil
Wiltshire et al.	8/F	14950	118	87.3	2.9	primary	nil
Elias	22/M	4000	135	1280	0.8–2	primary	nil
Solter et al.	33/M	12560	125	1891.4	1.2	primary	nil
Lau et al.	40/F	30779	106	832	5.8	primary	nil
Muir et al.	22/M	> 25000	110	4400	2.9	primary	severe hypothyroidism
Martin-Campagne et al.	9/M	1043	120	1250	1.4	primary	autoimmune polyglandular syndrome type 2
Tahir et al.	68/F	5000	120	NR	NR	primary	hypothyroidism
Foppiani et al. (current case)	26/M	6652	113	1500	0.8	primary	nil
Robillon et al.	31/F	21800	NR	22	8	secondary	panhypopituitarism
de Witte et al.	48/M	438	normal*	low*	low*	secondary	nil
Sayarlioglu et al.	58/F	> 40000	94	NR	2.1	secondary	panhypopituitarism
Oki et al.	52/M	11902	118	23	2.6	secondary	nil
Rezvanfar et al.	30/F	50000	137	NR	4	secondary	panhypopituitarism
Foppiani et al.	66/F	4250	123–127	low*	2.5	secondary	panhypopituitarism
Soresi et al.	64/F	1377	121	17	1.4	secondary	panhypopituitarism
Kennedy et al.	55/F	62270	137	< 10	0.6	secondary	nil
Komatsu et al.	67/F	6968	118	3.1	0.7	secondary	nil
Zhou et al.	22/M	5898	126	3.4	1.2	secondary	panhypopituitarism

NR, not reported.
* value not reported.

at least three months; indeed at that time the patient had been evaluated in another hospital and the association of severe hyponatremia, potassium levels in the upper normal range, vomiting and hypotension was found. The combination of these biochemical and clinical findings, although not diagnostic, were highly suggestive of adrenal failure. This emphasises that a high clinical suspicion is mandatory, in order to avoid misdiagnosing AD.

In summary, our case reveals that the association of hyponatraemia and rhabdomyolysis may be the initial finding of an unknown AD, which can be life-threatening and therefore requires prompt diagnosis and proper treatment.

CONFLICT OF INTEREST

Informed consent was obtained by the patient. "The authors declare that there is no conflict of interest regarding the publication of this paper." No grants or funds were received.

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