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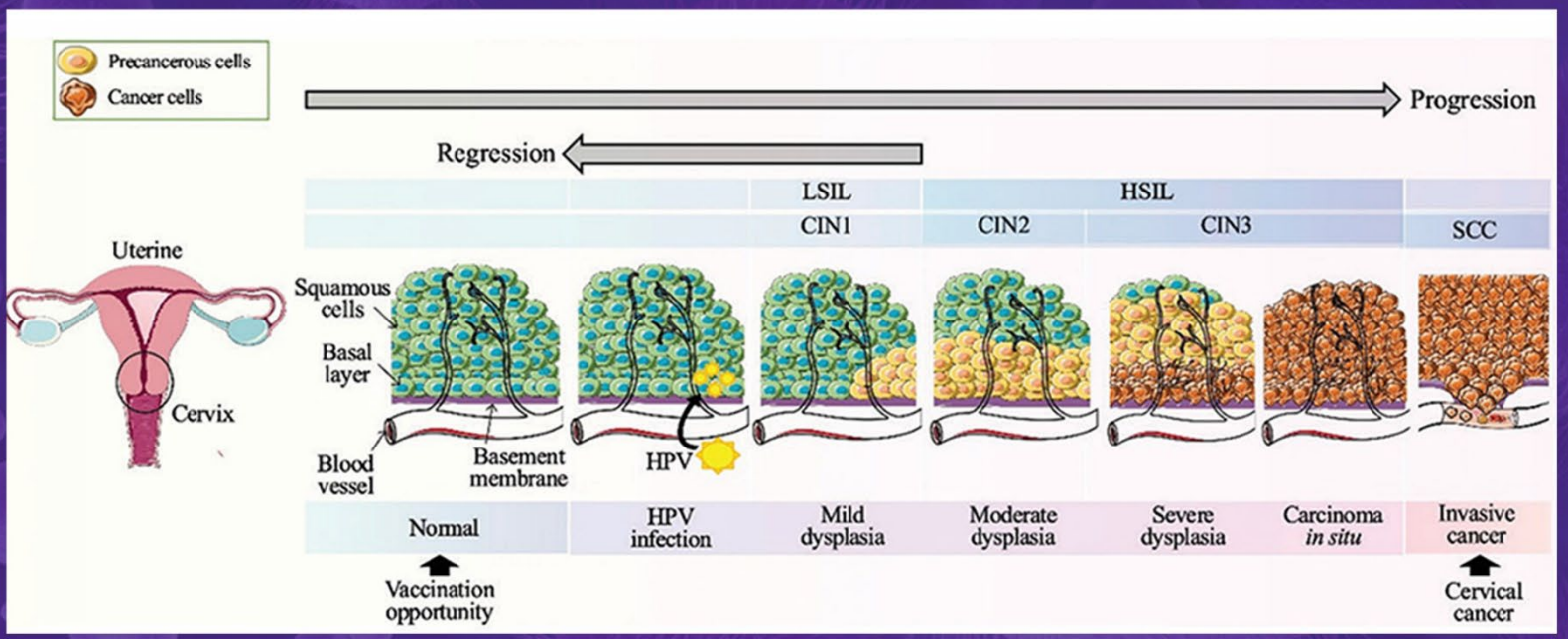

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Journal of Clinical and Translational Research

REVIEW ARTICLE

Clinical review and insights into lateral patellar instability in deep flexion

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Abstract

Background: Lateral patellar instability in deep knee flexion is a poorly understood and underreported condition that differs etiologically and biomechanically from the more common instability near extension. **Aim:** This paper presents a comprehensive review combined with clinical experience on lateral patellar instability in deep flexion, along with insights into the underlying anatomical and biomechanical characteristics. **Methods:** A systematic literature search was performed using the terms "patellar instability" and "knee flexion." Inclusion criteria included original studies, book chapters, and reviews in English, whereas computational or biomechanical studies were excluded. In addition, clinical experience from cases was incorporated into the considerations and assessments. **Results:** Nine studies met the selection criteria consisting of three case reports, three case series, a book chapter, and two reviews. The analysis of clinical, anatomical, biomechanical, and kinematic factors in patients with lateral patellar instability in deep flexion did not reveal reliable arguments for the same etiological factors causing instability near extension. Instead, factors such as changes in the shapes of the lateral and medial condyle during knee flexion, variations in the shape of the lateral femoral condyle, terminal sulcus or false groove, short extensor muscles, contractures of soft tissues lateral to the patella, and laxity of the medial ligaments play significant roles in flexion instability. **Conclusion:** Lateral patellar instability in deep flexion is a rare but severely disabling condition that often begins at a younger age. The etiological factors leading to deep flexion instability differ from those causing patellar instability near extension, necessitating a clear distinction between these two types of patellofemoral instability. Accordingly, surgical treatment should address all documented etiological factors for flexion instability and involve a combination of procedures. **Relevance for patients:** Accurate differentiation between lateral patellar instability in deep flexion and instability near extension is essential for devising effective treatment strategies.

Keywords: Patellar instability; Flexion; Etiology; Anatomy; Biomechanics; Treatment

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1. Introduction

The American Orthopedic Society for Sports Medicine/Patellofemoral Foundation Patellofemoral Instability Workshop defined patellofemoral stability as "constraint by passive soft-tissue tethers and chondral/bony geometry that, with muscular forces, guide the patella into the trochlear groove and keep it engaged within the trochlear groove as the knee flexes and extends" [1(p.1)]. Patellofemoral instability is a deficiency of these

constraints to allow the patella to subluxate partially or completely from its position in the femoral trochlea.[1] In general, two types of lateral patellar instability are described: (i) instability close to extension or beginning flexion <45° and (ii) instability of the patella in deep flexion >45°.[1,2] Although instability in early flexion is a commonly known type of lateral patellar instability, there is still a general lack of information, and very little literature is available on instability in deep flexion.[2-4] In addition, most studies report a combination of both types of instabilities.[3] Patellar instability in deep flexion represents a habitual patellar dislocation and must be differentiated from recurrent patellar instabilities. It occurs only within the range of 50° – 80° of knee flexion, with the patella remaining centrally stable in the proximal trochlea near or close to full extension.[2] Therefore, understanding the close relationship between anatomy and patellar kinematics during deep flexion is necessary to assist clinicians in selecting the most effective treatment to address the pathological factors contributing to this form of instability.[2-8]

Various morphologic abnormalities, such as trochlear dysplasia (present in 85% of cases), patella alta, increased tibial tubercle-trochlear groove (TT-TG) distance, rotational abnormalities, external tibiofemoral rotation, and soft tissue abnormalities, are well-known factors causing lateral patellar instability near full extension.[3-8,14-18] In contrast, relatively little attention has been given to the predisposing factors that trigger instability in deep flexion. The same factors contributing to instability near extension (unusually patella alta, trochlear dysplasia grade C or D, increased TT-TG distance, or a combination of these factors) are commonly described as etiological features.[19] However, other potential etiological factors remain largely unaddressed.

Given these considerations, it is essential to clearly distinguish between lateral patellar instability in deep flexion and instabilities near extension. This requires a comprehensive evaluation of anatomy, biomechanics, and the kinematics of flexion instability to better understand the changing forces acting in all three-dimensional planes.

2. Methodology

A systematic literature search was performed through PubMed registries in 2024 according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines[20] using the terms "patellar instability" and "knee flexion" (Figure 1).

Inclusion criteria were original studies, book chapters, and review articles in English. Computational or biomechanical studies, abstracts, and technical notes were excluded. Studies of all evidence levels were included. In addition, the references of all included studies were analyzed, and the full text of all articles was reviewed. Furthermore, clinical experience from cases was incorporated into the considerations and assessments.

3. Results

Nine studies were selected based on the criteria for inclusion in the review. These studies comprised three case reports, three case series, a book chapter, and two reviews (Table 1). The full texts of these articles, related to patellar instability and flexion, were analyzed.

3.1. Anatomy

The patellofemoral gliding mechanism is determined by the three-dimensional shapes of the trochlear groove and patella. The articular part of the trochlea consists of the lateral and medial facets of the femoral sulcus and exhibits different anatomical aspects in the proximal-distal, mediolateral, and anteroposterior directions.[21-23] The normal trochlea deepens gradually from proximal to distal and is longer on the lateral side and shorter medially.[2,23] It begins at the anterior cortex of the distal femur and extends to the femoral notch.[24] The lateral and the medial facet are separated by the trochlear groove. In the anteroposterior direction, the lateral trochlear facet is typically higher than the medial condyle, engaging the patella in the extended knee (Figure 2).[21,22,25] This anatomical configuration allows the lateral facet to resist forces applied by the quadriceps angle effect in extension.[25] In contrast, with increased knee flexion, the medial facet becomes higher, with a crossing point of around 50° of flexion (Figure 3).[25] As a result, lateral resisting forces decrease in deep flexion due to the lowered inclination, causing the patella to track more laterally.[15,25-27]

In this context, the anteroposterior length and depth of the terminal sulcus are essential. The terminal sulcus is a shallow groove that separates the patellar and tibial articular surfaces of the femur.[28,29] Located laterally to the intercondylar notch, it has a triangular shape with an indentation up to 1.5 mm depth, which can vary individually.[30] It does not extend more than 10 mm posterior to the Blumensaat line.[28,31]

In addition, the lateral femoral condyle shape can change in patients suffering from patellar instability in deep flexion. Persistent abnormal motion of the patella in higher flexion at a young age, caused by a large quadriceps vector, excessive lateral patellar tilt, skeletal deformations, or rotational abnormalities, may form a false sulcus in the middle of the lateral femoral condyle, resulting in subsequent flattening.[2,4] Furthermore, the shape and thickness of the bone and cartilage of the lateral condyle in

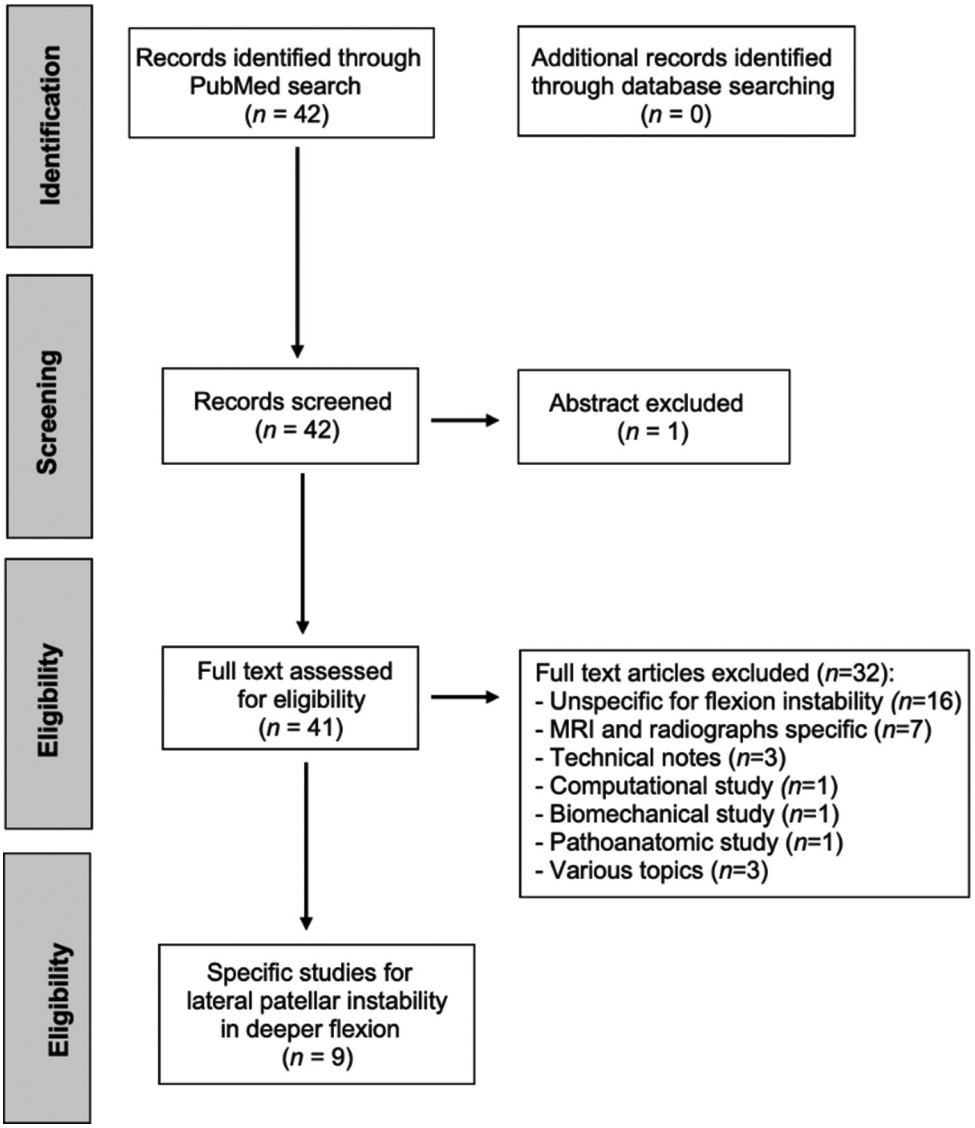

Figure 1. A flowchart shows the inclusion and exclusion criteria of the review according to the preferred reporting items for systematic reviews and meta-analysis guidelines[20]

the sagittal and coronal planes are not constant.[25] Skeletal dysplasia, such as nail-patella syndrome, is characterized by a short distal lateral femoral condyle and a decreased anterior distal lateral femoral angle.[25,32]

The congruity between the patella and the trochlea, along with the condition of the surrounding soft-tissue structures, is vital in deep flexion.[2,15] The medial patellar ligaments provide stability to the patellofemoral joint, with the medial patellofemoral ligament serving as the primary passive stabilizer to lateral translation of the patella in early knee flexion, contributing 50 – 60% of the restraint.[33,34] In addition, the medial patellotibial ligament and the medial patellomeniscal ligament are essential during final knee extension, counteracting quadriceps contraction.[33-35] In deeper knee flexion, the contribution of the medial patellotibial ligament and the medial patellomeniscal ligament as secondary restraints increases, providing up to 46% of the restriction on lateral patellar translation at 90°.[33,36,37]

3.2. Biomechanics

At the onset of knee flexion, the femoral condyles shift posteriorly relative to the tibia due to the rolling-gliding mechanism.[30,38-40] The lateral femoral contact point rolls back during flexion, while the medial femoral condyle remains relatively fixed.[40-42] This differential rolling-gliding mechanism causes the femur to rotate externally and the tibia to rotate internally.[40-42]

Table 1. Selected studies and data for lateral patellar instability in deep flexion

Author	Year	Study design	Number of subjects	Age at onset of symptoms	Clinical presentation	Age at surgery	Recommended surgery
Bergman & Williams[5]	1988	Case series	35 (18 males and 17 females)	5 – 12 years old	Quadriceps muscle contractures and odd-looking knee	6 – 15 years old	Lateral release, medial plication, patellar tendon, or patella transfer
Eilert[60]	2001	Case report	Two (females)	2 months – 6 years old	Persistent/obligatory lateral dislocation	2 months or 6 years old	Recentering patella
Joo *et al.* [64]	2007	Case series	Six (females)	At the start of walking	Ligamentous laxity and aplasia trochlear groove	6.1 years old (4.9 – 6.9 years old)	Lateral release, semitenodesis, proximal tube transfer, or patellar tendon transfer
Shen *et al.* [65]	2007	Case series	12 (five females and seven males)	10.8 years old (average)	Patellofemoral pain, weakness, crepitus, and effusion	25.4 years (18 – 50 years old)	Lateral release, medial retinaculum advancement, or anteromedial tibial tubercle transfer (osteotomy for valgus)
Biedert[2]	2012	Case report	Two (one male and one female)	14 and 11 years old	Pain, crepitus, weakness, and restricted range of motion.	21 and 24 years old	Re-medialization tibial tubercle, medial patellofemoral ligament reconstruction, or raised lateral trochlea
Batra & Arora[3]	2014	Review	-	Children	Lateral soft tissues and quadriceps muscle contractures	25.4 years old	Excessive lateral release, advancement of vastus medialis obliquus muscle, or distal realignment
Sanchis-Alfonso[19]	2015	Review	-	-	Increased tension in extensor muscles	-	Lengthening lateral release, quadriceps muscle, rectus lateralis tendon, medial patellofemoral ligament reconstruction, or raised lateral condyle
Mittal[67]	2020	Case report	10 (two females, eight males)	5 – 9 years old	Hypoplastic patellae	6.4 years old	Excessive lateral release or Goldthwait Roux tibial tubercle transfer
Weitz[4]	2020	Book	-	At the start of walking	Extensor contractures, muscles, dysfunction, and false sulcus	-	Lengthening vastus lateralis muscle tendon, medial patellofemoral ligament reconstruction, or lifting false groove

Simultaneously, the iliotibial tract glides posteriorly across the rotational axis and lateral femoral condyle, changing the resultant force vector to lateral and posterior aspects. The iliotibial tract has direct connections to the lateral retinaculum.[43,44] The superficial oblique retinaculum extends from the iliotibial tract, along with fibers of the vastus lateralis tendon, to the lateral aspect of the patella and the patellar tendon (Figure 4).[43] The deep transverse retinaculum fibers connect the fascia lata directly to the lateral patella. Functionally, both parts of the retinaculum are dynamized by the vastus lateralis, tensor fasciae lata (iliotibial tract), and gluteus maximus muscle. This causes a posterolaterally oriented force on the patella during knee flexion.[43,45] In addition, tightness in the fascia lata and the iliotibial band increases the quadriceps angle, thus exacerbating the lateral force vector.

Close to extension, the lower third of the articular cartilage of the patella contacts the upper trochlea with its high lateral facet and inclination.[46,47] At 45° of flexion, the midportion of the patella is in contact with the mid

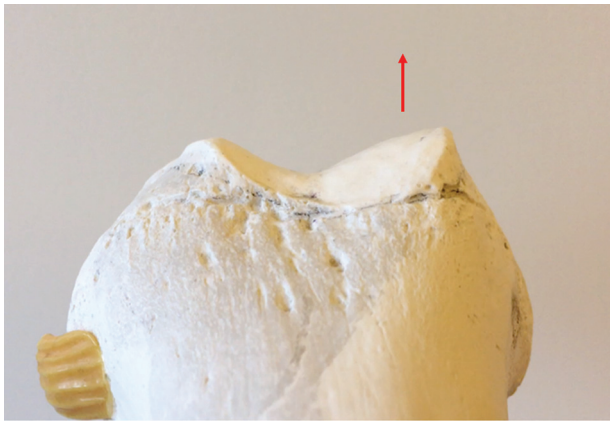

Figure 2. Axial view of the right knee. During extension, the lateral trochlear facet (red arrow) is normally higher than the medial condyle. Source of image by the author.

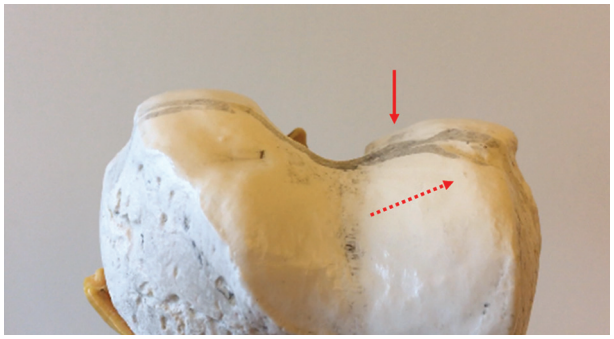

Figure 3. Anterior view of right knee. In flexion, the medial facet becomes more, and the lateral facet becomes less prominent. The red arrow indicates lateral condyle with terminal sulcus, while the dotted red arrow indicates the direction of lateral patellar instability in deep flexion.
Source of image by the author.

femoral trochlear articular cartilage, whereas at 90°, the proximal third of the patellar cartilage contacts the lower articular area of the trochlea.[1,2,30,39,46,47] Between 45° and 90° of flexion, the patella makes contact with the terminal sulcus. In deep flexion, the reduced thickness of the upper patellar cartilage results in articulation with the lateral femur, which increases the tendency for the patella to move laterally.[46,48]

In addition, the quadriceps and patellar tendons pull almost in the opposite direction near the extension. The patellofemoral joint reaction force is low in this position. As the knee flexes, the angle between the quadriceps and patellar tendons decreases in the sagittal plane, causing higher patellofemoral joint reaction force.[25] Consequently, the tension in the patellar tendon is lower (about 70%) than in the quadriceps tendon.[49,50] This increase in quadriceps tendon tension, combined with the changing shape of the lateral femoral condyle and the roll-back mechanism of the femur, represents a significant biomechanical factor

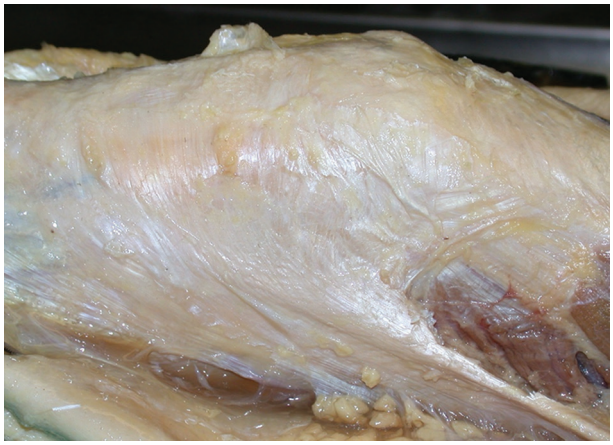

Figure 4. Lateral view of the left knee. Connections from the iliotibial tract to the superficial lateral retinaculum, patellar tendon, patella, and vastus lateralis tendon.
Source of image by the author.

contributing to patellar instability in deeper knee flexion. The biomechanical sequence of patellar instability in deep flexion is summarized in Table 2.

3.3. Common instability factors

Three factors are relevant for symptomatic patellar instability: trochlear dysplasia, patella alta, and TT-TG distance.[30]

3.3.1. Trochlear dysplasia

Trochlear dysplasia is defined as a geometrical abnormality of the shape, depth, and length of the trochlear groove, mainly at its proximal part.[1,2,10-13,15,16,24,31,46,51] Different forms of trochlear variations, such as decreased depth, large sulcus angle, decreased inclination of the lateral facet, flat trochlea, trochlear bump, and hypoplasia of the medial trochlea, contribute to decreased bony stability in the trochlear groove. As a result, patellar engagement in the trochlea is insufficient during the early stages of knee flexion, leading to lateral instability. In summary, patella instability caused by common trochlear dysplasia typically occurs in extension and early knee flexion but not necessarily in deep flexion beyond 45°. In addition, intraoperative findings in patients with patellar instability in deep flexion have shown a normal trochlea in its proximal extent.[2] Therefore, common trochlear dysplasia does not appear to be a significant factor in flexion-related instability.

3.3.2. Patella alta

Patella alta is characterized by a more proximal position of the patella relative to the femur, the trochlear groove, or the tibia, which may lead to decreased bony stability.[9,16,52] The articular patellofemoral contact area is reduced with

Table 2. The biomechanical sequence of lateral patellar instability in deep flexion

Angle	Biomechanical sequence
<45° (stable patella)	• The lateral facet of the trochlea is the most prominent, and the inclination is high. • The quadriceps tendon and patellar tendon pull almost in the opposite direction. The patellofemoral (PF) joint reaction force (JRF) is low. • The iliotibial tract, with its connections to the lateral patellar structures, runs anterior to the rotational axis, resulting in a low laterally oriented force vector.
>45° (dislocating patella)	• The medial facet of the trochlea becomes more, and the lateral facet becomes less prominent with lowered inclination, resulting in decreased lateral resisting forces. • The lateral femoral contact point rolls back, causing an external rotation of the femur. • The iliotibial tract glides posteriorly across the lateral femoral condyle, changing the resultant force vector and exerting a posterolateral force on the patella. • The smaller upper part of the patella has increasing contact with the terminal sulcus/false groove/ dysplastic lateral femoral condyle, and the osteochondral stability decreases. • The tension in the quadriceps tendon becomes higher than in the patellar tendon, resulting in a higher PF JRF. • Contractures/fibrosis of lateral soft-tissue structures and quadriceps tendon cause increased laterally oriented forces acting on the patella.

decreased patellotrochlear cartilage overlap.[9,16,52] As a result, the patella alta prevents proper engagement of the proximal trochlea during extension and early flexion. Therefore, patella alta is considered a potential risk factor for patellar instability close to extension.

Biomechanically, the flexion angle at which a patella alta becomes engaged by the trochlear groove is increased.[16] In deep flexion, the patella is more securely engaged in the trochlear groove, enhancing stability.[16] The patella only contacts the terminal sulcus at very high flexion, not at the angles where flexion instability occurs. In summary, patella alta is not a biomechanically significant factor contributing to patellar instability in deep flexion.

3.3.3. Lateralization of the tibial tubercle

Excessive lateralization of the tibial tubercle is considered a major predisposing factor for patellar instability. Two common measurements used to assess the position of the tibial tubercle are the TT-TG distance and the distance from the center of the patellar tendon attachment on the tibial tuberosity to the medial border of the posterior cruciate ligament attachment on the tibia (TT-PCL).[40,51,53,54] The normal range of TT-TG values measured in extension has been reported to be 9.3 – 16.1 mm on computed tomography scan (CT)[51] and 8.9 – 11.1 mm on magnetic resonance imaging (MRI).[40,53] Cutoff values commonly measured in extension more than 20 mm are considered pathologic.[40,54-56] A TT-PCL distance of <24 mm is considered normal.[55]

However, the angle of knee flexion during imaging plays a critical role in accurately measuring both the TT-TG and TT-PCL distances for assessing patellar instability. Both TT-TG and TT-PCL distances increase significantly during the final knee extension due to the screw-home mechanism.[57,58] Tanaka *et al.*[57] described that the TT-TG distance decreased by approximately 1 mm with each 5° increase of knee flexion between 5° and 30° in patients with patellar instability.[40,57] Other authors confirmed that the TT-TG distance assessed in the axial plane decreased with greater flexion.[6,40,59] As knee flexion occurs, tibial internal rotation and tibial tubercle medialization reduce the lateral force vector of the quadriceps angle.[25] The lateral position of the tibial tubercle, as a relevant factor for patellar instability in extension, decreases with deeper knee flexion.[40-42] Thus, the anatomic location of the tibial tubercle does not appear to be a significant factor in patellar instability during deep flexion.

In summary, these biomechanical and clinical reflections indicate that other etiological pathologies associated with lateral patellar instability in deep flexion should be considered when determining appropriate surgical treatment.

3.4. Clinical evaluation

This rare lateral patellar instability in deep flexion can be identified through physical examination and confirmed with radiographs, CT, and MRI.[2] This condition typically begins at a younger age and is often well tolerated for an extended period.[4,5,60] Over time, however, dysfunction and instability may result in difficulties with daily activities and running.[5,60] A comprehensive history, including various symptoms, unsuccessful treatments (whether conservative or surgical), functional disability, and a thorough physical examination, are essential factors for diagnosis.

3.4.1. Clinical assessment

The patellofemoral joint and surrounding soft tissue structures are thoroughly examined, including instability tests, tightness, patella gliding during the whole range of motion, muscle conditions, and contractures. Typically, the patella escapes laterally only in deep flexion beyond 45° (Figure 5A).[2,5] Close to extension, the patella is stable and well-engaged (Figure 5B). The patella subluxates or dislocates laterally each time the knee is flexed, but full flexion can still be achieved when the patella is

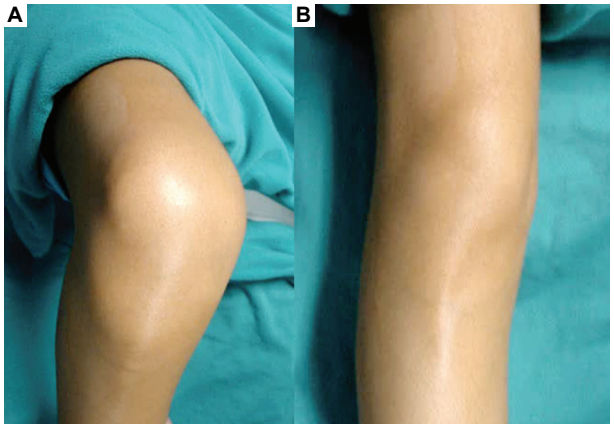

Figure 5. Lateral patellar instability in deep flexion in the right knee. (A) Dislocated patella in higher flexion and (B) relocated patella in extension.
Source of image by the author.

dislocated.[2,3,5] The most common clinical presentation is an unusual "odd-locking knee" appearance.[2,5] Shortened extensor muscles and contractures in the iliotibial band, vastus lateralis, and lateral fibers of the rectus femoris muscle are often observed. Pain is an uncommon symptom.[3] However, dysfunction and instability lead to difficulty in daily activities and running.

3.4.2. Imaging

Imaging consists of radiographs (standing long anteroposterior, true lateral, and axial views), CT scans, and/or MRI.[2] Radiographs document the lateral patellar subluxation/dislocation in deep flexion (Figure 6). Common radiological findings of proximal trochlear dysplasia, such as supratrochlear spur, crossing sign, and trochlear prominence, are absent (Figure 7).[2] In contrast, imaging often shows a flattened or dysplastic lateral femoral condyle. The distal lateral femoral angle may also appear pathologic, and the distal lateral femoral condyle may be too short.

CT scans (axial and three-dimensional imaging) confirm the normal shape of the proximal trochlea. They also highlight the decreased inclination of the distal lateral trochlear facet and the pathologic form of the lateral condyle with lateral subluxation/dislocation (Figure 8A and B). Contrast-enhanced CT can further visualize a false groove in the lateral femoral condyle.[4] When there is suspicion of variations in the lateral femoral condyle, three-dimensional CT is the preferred imaging modality.

MRI (two-dimensional and three-dimensional) is another reliable modality to assess the bony and cartilaginous morphology of the knee, particularly the trochlea.[22,61,62] MRI findings in patients with patellar instability in flexion revealed injuries to the lateral femoral

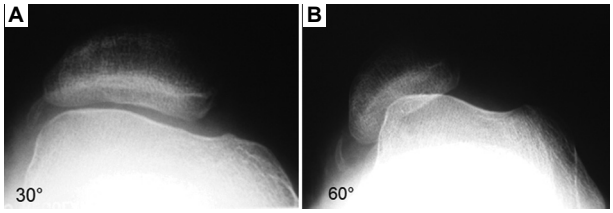

Figure 6. Radiographs of the right knee. Axial radiographs show (A) well-centered patella at 30° and (B) lateral patellar dislocation at 60°.
Source of image by the author.

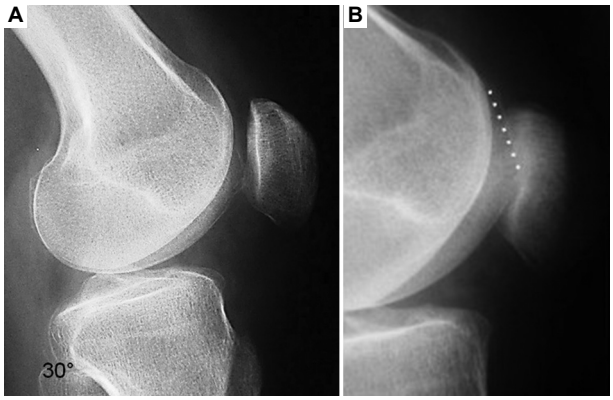

Figure 7. Sagittal radiographs show (A) normal trochlea and patellar height at 30° and (B) lateral patellar dislocation at 60°. The dotted line refers to the flattened mid-femoral lateral condyle.
Source of image by the author.

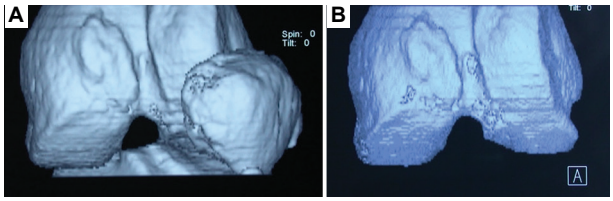

Figure 8. Three-dimensional computed tomography scans of the left knee. (A) Lateral patellar dislocation in 60° of flexion (condition after an unsuccessful attempt at proximal deepening trochleoplasty). (B) Dysplastic and flattened mid-femoral lateral condyle (same patient as Figure 7A without patella).
Source of image by the author.

condyle at or immediately anterior to the terminal sulcus,[63] contractures of the quadriceps muscle, most common in the vastus lateralis muscle with signs of fibrosis, and rarely in the iliotibial band or rectus femoris.[3,63] In addition, MRI can also show elongation, thinning, and scar tissue formations in the medial patellar ligaments.

3.5. Surgical treatment

The etiological factors and underlying pathologies causing lateral patellar instability in deep flexion differ from those for instabilities close to extension (Table 3). Hence, the surgical treatment is not the same and must be individually adapted.[2,5]

Operative treatment for flexion instability should address all documented etiological factors and typically involve a combination of procedures.[2,5,8,64-66] Two types of surgical reconstruction are described: (i) soft-tissue reconstruction, including lateral and medial approaches,[3,67,68] and (ii) soft–tissue reconstruction combined with bone reconstruction, such as elevation of the lateral femoral condyle.[2,4] Contractures of the soft tissues lateral to the patella are among the most important contributing factors and must be addressed during surgery (Table 4).[5,8,69] Therefore, lengthening and/or releasing all involved structures is the first step in the reconstruction process.

The role of quadriceps tendon lengthening remains a topic of discussion.[4,64] Several quadricepsplasty techniques (i.e., sliding lengthening plasty of the lateral half of the distal quadriceps tendon, 4-in-1 quadricepsplasty, and subchondral quadriceps realignment) have been recommended, particularly for treating congenital patellar dislocation.[4,64,67,68] A 4-in-1 quadricepsplasty, with or without lengthening, may be necessary in rare cases with more severe forms of flexion instability to correct the externally rotated and shortened quadriceps mechanism.[3,64,68] Z-lengthening of the quadriceps tendon can be performed to restore its proper length.[68] However, normal patellar tracking has also been reported without requiring quadriceps tendon lengthening.[2,64]

Table 3. Pathological factors for lateral patellar instability in deep flexion

Factors (most common in combination)[2-5,7,25,64]
Changing shapes of the lateral and medial condyle during knee flexion
The shape of the terminal sulcus
False groove to the middle of the lateral condyle
Dysplasia of the lateral femoral condyle
Quadriceps contracture
Large quadriceps vector
Contractures/fibrosis of lateral soft tissue structures (vastus lateralis or iliotibial band)
Ligamentous laxity (medial patellofemoral ligament, medial patellotibial ligament, and medial patellomeniscal ligament)
Genu valgum
Torsional abnormalities (increased femoral antetorsion or external tibial torsion)

Table 4. Combination of recommended procedures

Type	Combination of recommended procedures[2-5,8,33,35-37,64,68,70,71]
Basic	• Adhesiolysis of lateral scar tissue formations and extensor apparatus • Lengthening/release of contracted lateral soft-tissue structures (vastus lateralis, iliotibial tract, and retinaculum) • Reconstruction of medial patellofemoral ligament
Optional	• Elevation of the hypoplastic lateral femoral condyle, terminal sulcus, and false groove by incomplete osteotomy • Lengthening of the extensor apparatus (Z-lengthening) • 4-in-1 quadricepsplasty technique • Tibial tuberosity transposition • Patellar tendon transfer • Additional medial patellotibial ligament reconstruction

Biomechanically, the decreased resisting forces of the lateral femoral condyle are crucial in the reconstruction process.[2,4,25] Any dysplastic shape of the lateral femoral condyle, a pathologic terminal sulcus, and/or a false groove must be corrected to restore the proper morphology according to the proximal and distal spherical morphology if present. This correction is determined by the condition of the soft tissue after the release of contracted structures and the patellar stability during higher flexion. There is a clear indication for additional bone reconstruction if patellar instability in flexion persists after extensive release of the soft tissues.

3.5.1. Surgical technique

The procedure is performed under tourniquet control, with the dislocated patella exposed through a lateral incision (Figure 9A and B). The lateral adhesions, scar tissue formations, tight lateral bands, iliotibial tract, and vastus lateralis are released, and the retinacula (superficial and deep) are incised in two layers. If lateral patellar dislocation persists after extensive adhesiolysis, appropriate release, and temporary fixation of the medial structures using a clamp, bone reconstruction may be necessary. The mid-femoral and distal condyle are assessed for any existing osseous variations (Figure 10). If pathologies are present,

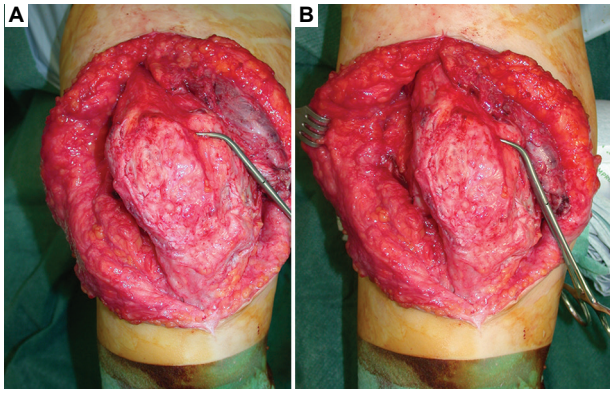

Figure 9. Anterior view of right knee. (A) Lateral dislocation of the patella with contractures of the lateral soft-tissue structures and excessive external rotation of the extensor apparatus. (B) Reposition of the patella with a clamp. Source of image by the author.

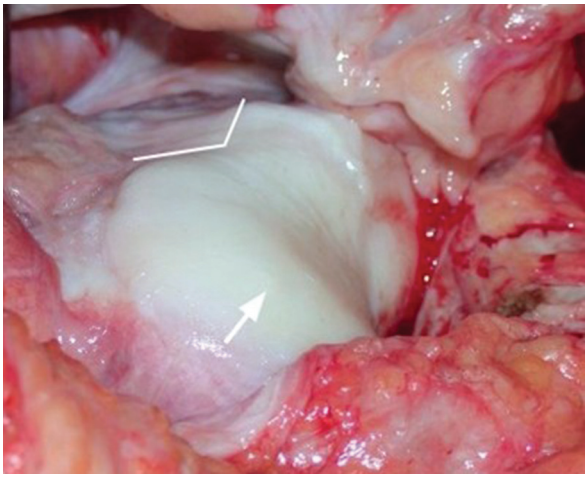

Figure 10. Antero-lateral view of the right knee. Intraoperative findings with normal proximal trochlea (white lines) and flattened mid-femoral condyle (white arrow).
Source of image by the author.

an incomplete subchondral osteotomy of the mid-femoral lateral condyle, including the terminal sulcus and false groove, is performed, followed by careful elevation with chisels (Figure 11).[2,4] The gap created by the osteotomy is filled and stabilized with cancellous bone harvested from the posterior lateral condyle. The amount of lifting depends on the present pathology. Raising the lateral femoral condyle can be safely done at any age without risking damage to the growth plates.[4] The final alignment should match the spherical shape of the proximal and distal lateral femoral condyle. After bone reconstruction, the medial patellofemoral and medial patellotibial ligaments should be assessed and reconstructed to recreate the medial soft tissue support (Figure 12).[33,36,37,70,71] Medial and lateral soft tissue balancing is the final surgical step.

Postoperatively, partial weight bearing with 10 kg is allowed for 6 weeks, with initiation of physical therapy and use of a continuous passive motion machine. The range of motion is gradually increased, but it should be adapted according to patient tolerance.

4. Discussion

Lateral patellar instability in deep flexion is characterized by habitual patella dislocation whenever the knee is flexed beyond 45°, with spontaneous repositioning upon knee extension.[3] Most cases occur at a younger age and are well tolerated for extended periods.[3-5]

This review, examining clinical, anatomical, biomechanical, and kinematics factors in patients with lateral patellar instability in deep flexion, found no conclusive evidence that the same etiologic factors causing instability close to extension are at play in deep flexion.

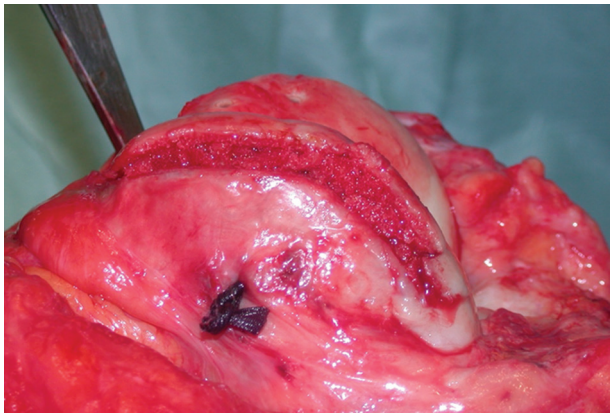

Figure 11. Elevation of the mid-lateral femoral condyle and impaction of cancellous bone. The center of the distal trochlea is secured with a suture (Vicryl, 5 mm) and/or smart nails.
Source of image by the author.

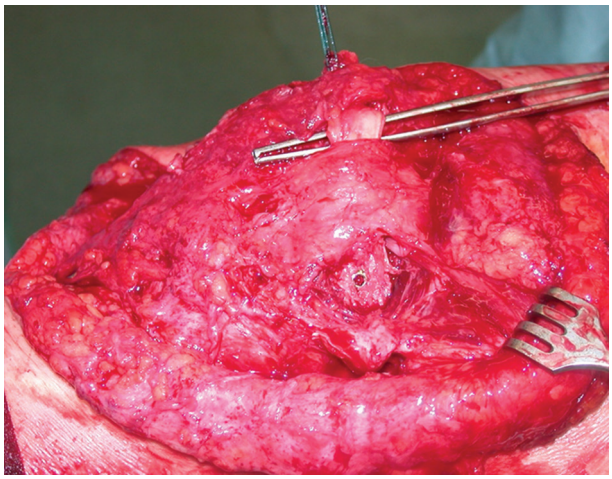

Figure 12. Medial view of the right knee. A quadriceps tendon graft is used for medial patellofemoral ligament reconstruction.
Source of image by the author.

Instead, factors such as altered shapes of the lateral and medial condyle, variations of the lateral condyle (flattened, false groove, and short distal), a large terminal sulcus, shortened extensor muscles, and contractures of the lateral soft-tissue structures play a significant role in flexion instability.[2-5,15,25,63] Sallay *et al.*[63] documented patellar instability in the range of 70° – 80°. MRI and arthroscopic findings revealed that the patella engages the terminal sulcus within this range of flexion, correlating with the onset of instability.[63] Contractures of the vastus lateralis and the iliotibial tract, in combination with quadriceps fibrosis, may cause lateral patellar instability in deep flexion. Bergman and Williams[5] concluded that habitual dislocation of the patella in flexion is due to contracture of different lateral elements of the quadriceps muscle.[5] However, extrinsic factors, such as femoral torsion, dysplasia of the lateral condyle, genu valgum, lateral placed

patellar tendon insertion, and ligamentous laxity, also play crucial roles in this condition.[5]

5. Conclusion

Lateral patellar instability in deep flexion is a rare yet severely disabling condition that typically begins at a younger age. The etiological factors differ from those associated with patellar instability near extension. Key contributors to flexion instability include changes in the shapes of the lateral and medial condyle during knee flexion, variations in the shape of the lateral femoral condyle, contractures of the vastus lateralis muscle, and iliotibial tract in combination with quadriceps fibrosis. Surgical treatment should address all documented etiological factors for flexion instability to recommend an appropriate combination of procedures for this condition.

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Conflict of interest

The author declares no competing interests.

Author contributions

This is a single-authored article.

Ethics approval and consent to participate

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Consent for publication

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Further disclosure

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ACCSCIENCE PUBLISHING

Journal of Clinical and Translational Research

REVIEW ARTICLE

A systematic review of immunogenicity and safety of influenza subunit vaccines and split vaccines

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Abstract

Background: Vaccination remains the most effective preventive measure against influenza. Current flu vaccines include split-virus, subunit, and live-attenuated vaccines. Comparing adjuvanted and non-adjuvanted subunit vaccines and split-virus formulations is essential to evaluate their immunogenicity (through geometric mean titers [GMTs] and seroprotection rates) and safety (adverse event rates). **Aim:** The aim of this study was to analyze the immunogenicity and safety of adjuvanted subunit vaccines, non-adjuvanted subunit vaccines, and split vaccines. **Methods:** A systematic search of the PubMed, Cochrane, and EMBASE databases was conducted, supplemented by manual searches. After two reviewers independently screened the articles, extracted the data, and assessed the quality, a meta-analysis was conducted with Stata 16.0 software. **Results:** Twenty-four studies were ultimately included in the analysis. The systematic review found that adjuvanted subunit influenza vaccines (IV), non-adjuvanted IV, and split IV all provided good protection. Based on the seroconversion rate and GMTs levels, adjuvanted subunit IV was overall superior to non-adjuvanted split IV. However, adjuvanted subunit IV had lower safety compared to non-adjuvanted IV and split IV. Non-adjuvanted IV displayed similar seroprotection rates to adjuvanted subunit IV, providing sufficient protection. **Conclusion:** Adjuvanted subunit IV offers better immunogenicity but has a higher incidence of adverse reactions. For individuals with impaired immune systems, it is recommended to use adjuvanted subunit IV for better protection. However, for the majority of the population, non-adjuvanted subunit IV is recommended to achieve sufficient seroprotection rates and better safety. **Relevance for patients:** The systematic review is helpful for guiding better vaccination strategies and improves public health outcomes.

Keywords: Influenza; Adjuvant; Subunit vaccines; Split vaccines; Immunogenicity; Safety

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1. Introduction

Influenza is a respiratory infection that affects all age groups and can cause critical complications, particularly among vulnerable individuals. Due to its high contagiousness

and potential for serious complications, it remains an important public health concern globally.[1] Most patients can recover within a week; however, influenza viral infection can be particularly severe for the elderly, children, pregnant women, and immunocompromised individuals.[2,3] With the global outbreak of the COVID-19 pandemic, public attention and demand for vaccine administration have been increasing. In addition to the hazards caused by the COVID-19 virus, people have also started focusing on the transmission and control of other respiratory diseases, particularly influenza.

Vaccine immunization is considered a highly effective measure for preventing influenza and reducing its impact on individuals and communities.[4] In China, the currently recommended groups for influenza vaccine (IV) administration include individuals aged 65 years and above, children and adolescents aged 6 months and above, pregnant women, individuals with chronic diseases, such as heart disease, diabetes, and respiratory diseases, healthcare workers, other high-risk occupational workers, as well as individuals who frequently come into contact with large crowds, such as students, teachers, service industry workers, and other high-risk groups.[5] The Advisory Committee on Immunization Practices advises IV for all individuals 6 months or older with no contraindications.[6] The influenza virus undergoes rapid mutations, and the effectiveness of the vaccine diminishes over time. Therefore, the vaccine formulation is updated annually based on recommendations from the World Health Organization and the Committee for Medicinal Products for Human Use in the European Union. At present, IVs include inactivated whole virus vaccines, split virus IVs, subunit vaccines, virus-like particle vaccines, live attenuated vaccines, and recombinant virus vector vaccines.[7] In China, the approved IVs mainly include split virus vaccines, subunit vaccines, and live attenuated vaccines.[8]

Vaccine safety assessment is crucial to ensure that individuals can confidently receive vaccines with minimal risk. Subunit IVs only contain highly purified hemagglutinin (HA) and neuraminidase components.[9] Clinical studies have demonstrated that subunit IVs have better safety profiles compared to whole virus IVs and split virus IVs, especially when administered with adjuvant to enhance immunogenicity.[10] Our analysis also considers the incidence and severity of adverse reactions following vaccination with subunit IVs compared to split virus IVs. Through systematic evaluation of data from various studies, we assess the overall safety of these vaccines and identify potential serious adverse events that may occur.

The split IV is currently the most commonly used IV in China.[1,11] Although research on the safety and immunogenicity of subunit IVs is limited, further studies are necessary to rationally promote and administer subunit IVs. A commonly used method to assess the immunogenicity and safety of subunit IVs is to conduct statistical analysis by combining data from previous relevant studies. This can be achieved through systematic and meta-analysis. By assessing indicators, such as geometric mean titers (GMTs), serum protection rates, seropositivity rates, and adverse events among participants who received subunit IVs, we can compare the reliability of non-adjuvanted subunit IVs, adjuvanted subunit IVs, and split vaccines. The present study aims to provide useful information for IV vaccination strategies, thereby guiding the promotion and use of IVs and increasing vaccination rates.

2. Methods

All methodological procedures in this study strictly adhered to the Meta-analysis of Observational Studies in Epidemiology reporting guidelines as shown in section S1 in the Supplementary File.

2.1. Search strategy

An extensive retrieval of relevant references was performed in electronic databases from August 1, 2013, to August 31, 2024. A cross-checking process was then employed to determine the studies that met the specified criteria. The databases searched included PubMed, Cochrane, and EMBASE. The search terms employed were "influenza," "vaccine," "immunogenicity," and "safety." The entire query phrases for each database can be found in "S2. Search strategy" in the Supplementary File. Furthermore, additional studies were identified by manual screening of the reference lists of relevant systematic reviews and meta-analyses.

2.2. Inclusion and exclusion criteria

As shown in Figure 1, the inclusion criteria were as follows: (i) clinical or retrospective studies investigating the immunogenicity, efficacy, or safety of IVs, including those with subunit IVs; (ii) participants consistently administered with the same IV type during the entire intervention process; (iii) participants belonging to a healthy population; and (iv) full-text articles published in English to provide comprehensive clinical information.

The exclusion criteria included: (i) study populations involving non-healthy individuals; (ii) studies that do not specify the type of vaccine used; (iii) studies that do not involve subunit IVs; (iv) duplicate publications; (v) other publications, including letters, comments, case reports, and editorials; (vi) non-English papers; and (vii) investigations without completed datasets.

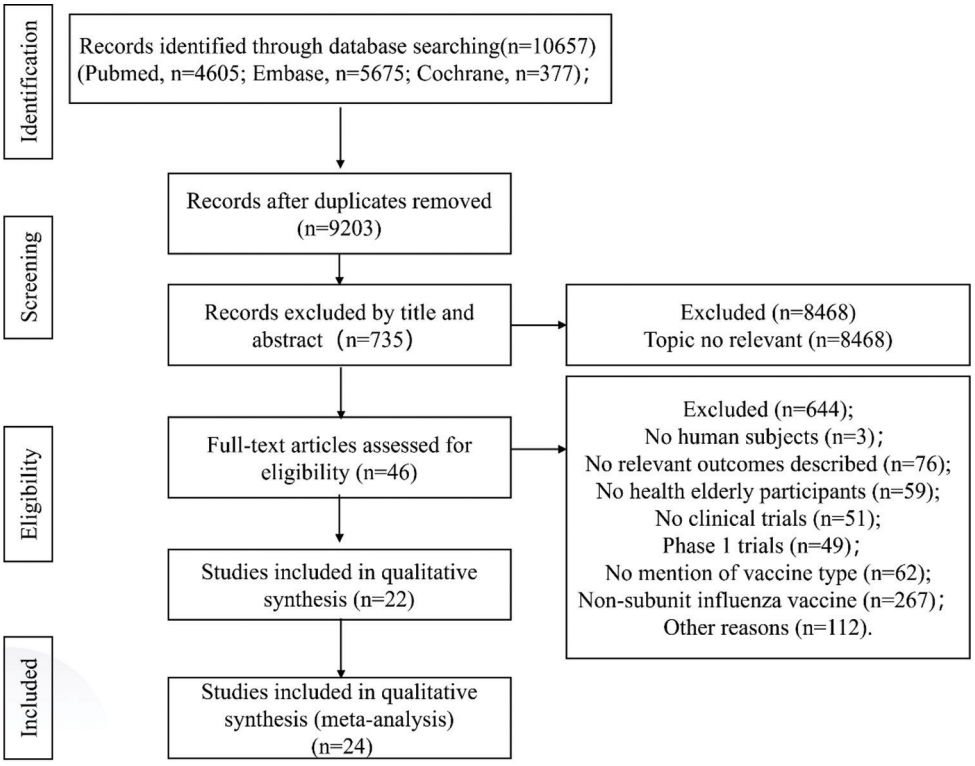

Figure 1. Flow chart of studies displaying the inclusion and exclusion of studies at every review step and reasons for exclusion

2.3. Study selection and data extraction

The methods of research article screening, assessment of methodological validity, data extraction, and cross-verification were performed through a double-blind review mechanism. An independent arbiter assisted in disambiguating any discrepancies and consulting the corresponding authors for additional evidence when necessary. The following details were extracted from the studies included in the analysis: first authorodological validity, data extraction, and cross-verification were performed through a double-blind review mechanism. An independent arbiter assisted in disambiguating any discreculture, use of adjuvant, safety data, and immunogenicity data.

2.4. Quality assessment

To gauge the quality of the studies that were included, the methodological reliability of the selected randomized controlled trials (RCTs) was assessed using the standards for quality evaluation outlined in the Cochrane Handbook for Systematic Reviews of Interventions 5.1.0. The risk of bias tool consists of seven domains: randomization protocol integrity (computer-generated sequences), allocation opacity, participant-personnel blinding integrity, masking fidelity of outcome assessment, missing data management, selective outcome reporting verification, and extraneous confounding factors. Bias susceptibility of individual investigations was classified as "low," "unclear," or "high." Study quality was independently assessed by two investigators, and any differences were settled through discussion.

2.5. Statistical analysis

This study conducted a statistical analysis of the immunogenicity data of subjects who received influenza vaccination. The primary outcome measures include seroprotection (defined as hemagglutination inhibition [HI] titer ≥40),[12] GMTs, and seroconversion. For subjects who were baseline negative (<1:10), seroconversion is defined as a HI titer of ≥1:40. For subjects who were baseline positive (HI ≥1:10),[13] seroconversion is defined as four times or greater increase in HI titer. Subgroup analyses were performed based on the common influenza virus strains.

This study conducted a statistical analysis on the safety data of subjects who received influenza vaccination, and the most prevalent local side effects (including injection site aches and swelling) and systemic side effects (including fever and muscle aches) associated with the vaccine were noted. Adverse events were classified by type, and the incidence rates and corresponding confidence intervals for each type were calculated. We performed a

subgroup analysis of adverse event types by comparing the occurrence rates of different types of adverse events between subunit IVs and split IVs. The heterogeneity of the outcomes was assessed utilizing the Chi-square test with the statistical significance threshold established at $\alpha = 0.05$. If $I^2 > 50\%$, it indicates significant heterogeneity. When significant heterogeneity exists, a random-effects model based on the Mantel-Haenszel method would be used to estimate the overall effect size. If no significant heterogeneity could be observed ($I^2 \leq 50\%$), the data would be synthesized using the fixed-effects model.

3. Results

3.1. Yield of search strategy

The preliminary search of the database generated 10657 studies. After screening the titles and abstracts, 735 studies were selected for whole-manuscript review. According to the inclusion and exclusion criteria, a total of 24 studies that met the standard were finally included. These studies would then be subjected to subsequent data examination and result integration.

3.2. Characteristics of included studies

The included studies in this research encompassed 17 countries and regions globally. A total of 48,943 participants was assessed in the immunogenicity and safety analyses. The vaccines examined in these studies included subunit IVs with adjuvant, subunit IVs without adjuvant, split IVs, and others. Among them, there were nine studies comparing subunit vaccines with split vaccines, four studies comparing subunit vaccines with or without adjuvant, four studies comparing subunit vaccines produced in eggs versus cell culture, and the remaining studies focusing on various other aspects of subunit IVs. Safety monitoring of the vaccinated population was conducted in 23 studies. In all included studies, the route of vaccination was intramuscular injection. More detailed information about each study is summarized in Table 1.

Most of the selected studies were evaluated as small likelihood of unfairness. Only a few studies did not specify the assignment method and were classified as ambiguous or at high risk of bias in the "selection bias" category. Since our detection results are not easily influenced by the blinding deficiency, the risks of "performance bias" and "detection bias" in this study are assessed as low. In addition, some studies were evaluated as having "attrition bias" due to participant loss. The quality appraisal of individual studies is available in S3. Quality assessment of the included studies (Table S1) is provided in the Supplementary File.

3.3. Immunogenicity

We conducted a systematic analysis of the GMTs, seroprotection rate, and seroconversion rate within 20 – 30 days after influenza vaccination. Among them, 18 studies reported results for seroprotection rate, 17 studies reported results for seroconversion, and 18 studies reported results for GMTs. We conducted subgroup analyses on three different types of IVs, including subunit vaccines with adjuvant, non-adjuvanted subunit vaccines, and split vaccines.

3.3.1. Seroprotection

We compared the seroprotection rates among different vaccine groups targeting four different influenza viruses (A/H1N1, A/H3N2, B/Victoria [BV], and B/Yamagata [BY]). According to the results in Figure 2, we found that these three types of vaccines displayed relatively good seroprotection rates against the four influenza strains. Particularly, for the two influenza A strains, H1N1 and H3N2, the seroprotection rates were both above 90% (H1N1: 94.99%, 95% confidence interval [CI]: 93.38 – 96.59%, I^2 = 96.40%; H3N2: 98.64%, 95% CI: 98.15 – 99.13%; I^2 = 81.1%). As for the influenza B strains, the seroprotection rates also exceeded 80% (BY: 89.93%, 95% CI: 86.32 – 92.53; I^2 = 97.6%; BV: 83.49%, 95% CI: 78.58 – 88.41, I^2 = 99.3%). Furthermore, the overall seroprotection rate of the non-adjuvanted subunit IV was similar to that of the adjuvanted subunit IV and the split IV across the four influenza strains.

3.3.2. Seroconversion

We further compared the seroconversion rates of different vaccine groups against the four influenza strains, 20 – 30 days post-vaccination. As displayed in Figure 3, the adjuvanted subunit IV exhibited significant superiority over the other two IVs for H1N1 and BY strains with seroconversion rates of 87.05% and 83.04%, respectively. As for H3N2, both the adjuvanted subunit IV and the split IV demonstrated seroconversion rates above 80%, outperforming the non-adjuvanted subunit IV. However, regarding the BV strain, the seroconversion rate of the adjuvanted subunit IV was 65.69%, which was slightly lower than that of the non-adjuvanted and split IVs at 70.31% and 74.81%, respectively.

3.3.3. GMT

We further compared the GMTs of different vaccine groups against the four influenza strains, 20 – 30 days post-vaccination. Figure 4 illustrates the GMTs of different vaccine groups for different influenza strains. For H1N1, the GMTs were 555.37 (448.38 – 666.36) for the adjuvanted subunit IV, 438.61 (340.77 – 536.45) for

Table 1. Description of included studies

First author	Year	Study design	Country	Vaccine produced in	Number of participants	Age	Duration	Adjuvant	HA per strain (μg/dose)	Type of virus strain	References
Scheifele *et al.*	2013	Prospective	Canada	Egg	911	≥65 years	2011 – 2012	MF59	15	IIV3	26
Knuf *et al.*	2015	Prospective	Germany, Belgium, Netherlands, Dominican Republic, Chile	Egg	666	3 – 17 years	2009 – 2011	MF59	3.75, 7.5, 15	H1N1	20
Vesikari *et al.*	2020	Prospective	Finland, USA, Philippines, Thailand	Egg	2,208	6 months – 22 years	2014 – 2016	MF59	15	IIV4	19
Beran *et al.*	2021	Prospective	Bulgaria, Colombia, Czech Republic, Estonia, Latvia, Lithuania, Malaysia, Philippines, Poland, Romania, Thailand, Turkey	Egg	6,790	≥65 years	2016 – 2017	MF59	15	IIV4	27
Yoo *et al.*	2018	Prospective	Korea	Egg	770	≥65 years	2010 – 2014	MF59	15	IIV3	28
Hartvickson *et al.*	2015	Prospective	USA	Cell	2,333	4 – 18 years	2013 – 2014	None	15	IIV4	29
Cruz-Valdez *et al.*	2018	Prospective	Mexico	Egg	287	6 – 72 months	2014 – 2015	MF59	7.5	IIV3	30
Knuf *et al.*	2014	Prospective	Germany, Belgium, Netherlands, Dominican Republic, Chile	Egg	330	6 – 35 months	2009 – 2011	MF59	3.75, 7.5, 15	H1N1	31
Basu *et al.*	2021	Prospective	India	Cell	480	≥18 years	2018 – 2019	None	15	IIV4	32
Diallo *et al.*	2018	Prospective	Senegal	Egg	296	6 – 71 months	2012 – 2013	Non-adjuvanted/ MF59	7.5, 15	IIV3	33
Zhang *et al.*	2022	Prospective	China	Egg	3,000	≥3 years	2020 – 2021	None	15	IIV4	34
Choi *et al.*	2017	Prospective	Korea	Cell	1,503	≥18 years	2013 – 2014	None	15	IIV3	35
Bart *et al.*	2016	Prospective	USA	Cell	2,680	≥18 years	2013 – 2014	None	15	IIV4/ IIV3	36
Vesikari *et al.*	2018	Prospective	Finland, USA, Canada, Italy, Poland, Spain, Philippines, Thailand, Taiwan (China)	Cell	10,644	6 months – 5 years	2013 – 2015	MF59	7.5, 15	IIV4/ IIV3	37
Mcbride *et al.*	2016	Prospective	Australia, New Zealand	Egg	15,044	18 – 65 years	2008 – 2010	None	15	H1N1/ IIV3	38
Frey *et al.*	2014	Prospective	Columbia, Panama, Philippines, USA	Egg	7,082	≥65 years	2010 – 2011	MF59	15	IIV3	39
Cowling *et al.*	2020	Prospective	Hong Kong (China)	Egg	3,283	65 – 82 years	2017 – 2018	MF59	15	IIV3	40
Loeb *et al.*	2021	Prospective	Canada	Egg	424	6 months – 6 years	2017 – 2019	MF59	15	IIV4/ IIV3	41
Eun *et al.*	2019	Prospective	Korea	Cell	454	6 months – 18 years	2014 – 2015	None	15	IIV4/ IIV3	42
Oh *et al.*	2018	Prospective	Korea	Cell	384	6 months – 18 years	2013 – 2014	None	15	IIV3	43

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

First author	Year	Study design	Country	Vaccine produced in	Number of participants	Age	Duration	Adjuvant	HA per strain (μg/dose)	Type of virus strain	References
Moehling *et al.*	2020	Prospective	USA	Cell	171	4 – 20 years	2018 – 2019	None	15	IIV4	44
Nolan *et al.*	2016	Prospective	USA, Australia, New Zealand, Philippines, Thailand	Cell/egg	2,055	4 – 17 years	2013 – 2014	None	15	IIV3	45
Diez-Domingo *et al.*	2016	Prospective	Spain, Italy	Cell/egg	430	3 – 18 years	2013 – 2014	None	15	IIV3	46
NCT02255409	2015	Prospective	Finland, Philippines, Thailand	Egg	1,601	12 months – 7 years	2016 – 2017	MF59	7.5, 15	IIV4	13

Abbreviations: IIV4: Quadrivalent inactivated influenza vaccine; IIV3: Trivalent inactivated influenza vaccine.

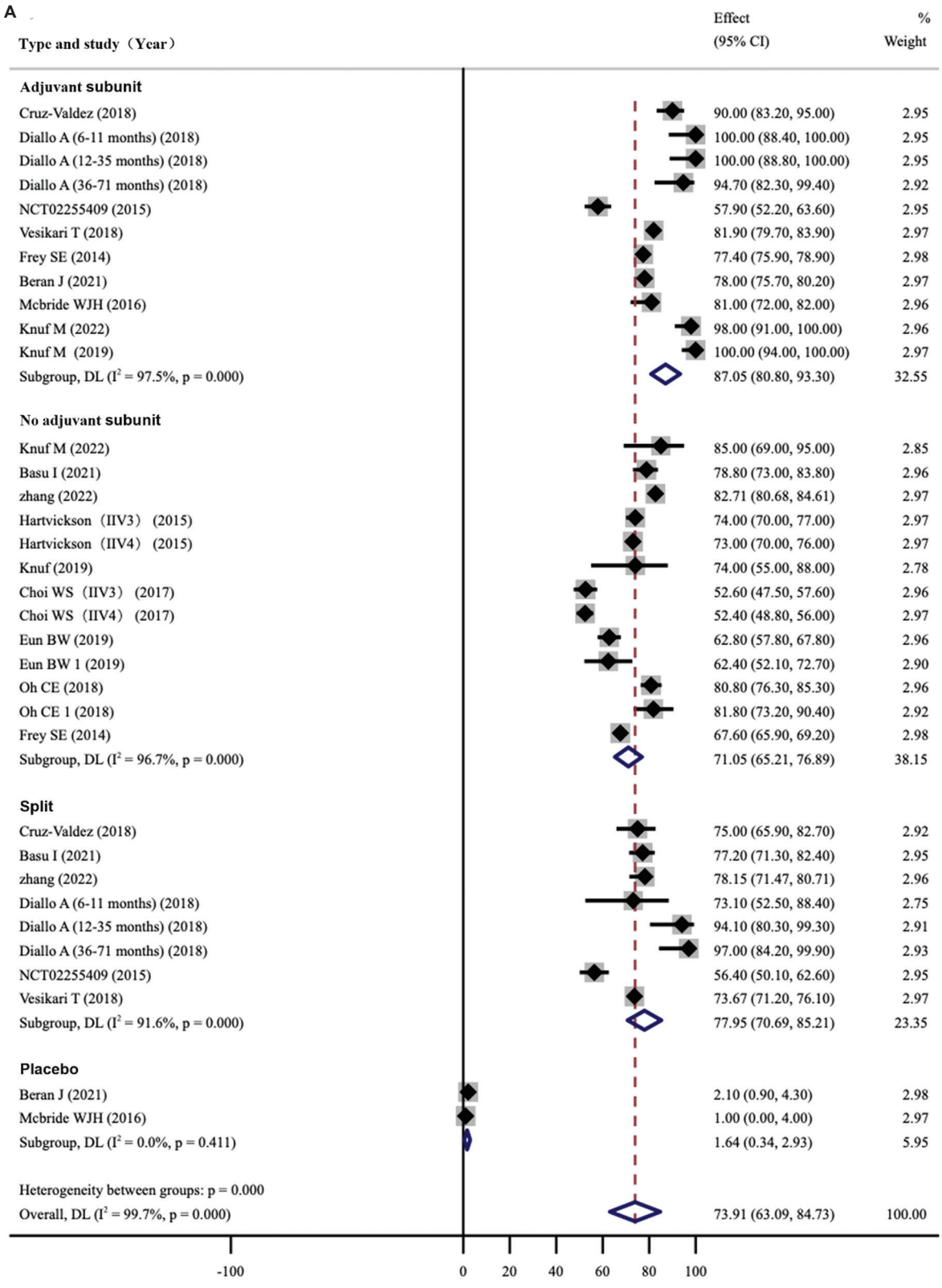
the non-adjuvanted IV, and 365.51 (257.96 – 473.07) for the split IV. Notably, the GMTs of the two subunit IVs were higher than that of the split vaccine. For H3N2, the adjuvanted subunit IV and the split IV demonstrated higher GMTs. For the influenza B strains, the GMTs of the non-adjuvanted subunit vaccine and the split vaccine were similar but significantly lower than that of the adjuvanted subunit vaccine. Overall, the adjuvanted subunit IV provided the highest GMTs, while the non-adjuvanted subunit IV and the split IV displayed similar GMTs.

3.4. Safety

An aggregate of 23 studies have documented the outcomes of side effects in relation to the administration of subunit IVs and split IVs. To compare the safety profiles of these two vaccine types, we extracted the most often reported local and systemic adverse events from the study data. A comprehensive statistical analysis was then conducted to assess and compare the safety profiles of these vaccine types.

According to the forest plot in Table 2, the incidence of adverse events was compared between subunit IVs (with adjuvant/without adjuvant) and split IVs. In the comparison between non-adjuvanted subunit IV and split vaccine, there was no remarkable difference in the total adverse events (relative risk [RR]: 1.04, 95% CI: 0.94–1.16, I^2 = 0%, p=0.917), but the sample size was limited, and further clinical studies are warranted for validation. In the comparison between adjuvanted subunit IV and split IV, the total adverse events were significantly higher for adjuvanted subunit IV compared to the split IV (RR: 1.42, 95% CI: 1.32 – 1.52, I^2 = 86.5%, p<0.001). It should also be noted that none of the studies reported any cases of mortality. Furthermore, the severity of adverse events was not taken into consideration in the analysis.

Further comparison was conducted regarding the impact of adjuvant use on the safety of subunit IVs. Figure 5 presents the forest plot of the occurrence rate of adverse events across two vaccine groups (with or without adjuvant). The results indicate that the total adverse event was significantly higher for the group receiving adjuvanted subunit IV compared to the group receiving non-adjuvanted subunit IV (RR: 1.54, 95% CI: 1.30 – 1.82, I^2 = 91.20%, p<0.001). This trend was also observed for both local adverse events (RR: 1.35, 95% CI: 1.20 – 1.51, I^2 = 62.3%, p=0.021) and systemic adverse events (RR: 1.39, 95% CI: 1.19 – 1.62, I^2 = 74.80%, p=0.001). Combining all the included studies, the occurrence rate of side effects was consistently higher for the group receiving adjuvanted subunit IV compared to the group receiving non-adjuvanted subunit IV.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Type and study (Year)
Effect (95% CI)
% Weight
Adjuvant subunit
Cruz-Valdez (2018) 90.00 (83.20, 95.00) 2.95
Diallo A (6-11 months) (2018) 100.00 (88.40, 100.00) 2.95
Diallo A (12-35 months) (2018) 100.00 (88.80, 100.00) 2.95
Diallo A (36-71 months) (2018) 94.70 (82.30, 99.40) 2.92
NCT02255409 (2015) 57.90 (52.20, 63.60) 2.95
Vesikari T (2018) 81.90 (79.70, 83.90) 2.97
Frey SE (2014) 77.40 (75.90, 78.90) 2.98
Beran J (2021) 78.00 (75.70, 80.20) 2.97
Mcbride WJH (2016) 81.00 (72.00, 82.00) 2.96
Knuf M (2022) 98.00 (91.00, 100.00) 2.96
Knuf M (2019) 100.00 (94.00, 100.00) 2.97
Subgroup, DL (I^2 = 97.5%, p = 0.000) 87.05 (80.80, 93.30) 32.55
No adjuvant subunit
Knuf M (2022) 85.00 (69.00, 95.00) 2.85
Basu I (2021) 78.80 (73.00, 83.80) 2.96
zhang (2022) 82.71 (80.68, 84.61) 2.97
Hartvickson (IIV3) (2015) 74.00 (70.00, 77.00) 2.97
Hartvickson (IIV4) (2015) 73.00 (70.00, 76.00) 2.97
Knuf (2019) 74.00 (55.00, 88.00) 2.78
Choi WS (IIV3) (2017) 52.60 (47.50, 57.60) 2.96
Choi WS (IIV4) (2017) 52.40 (48.80, 56.00) 2.97
Eun BW (2019) 62.80 (57.80, 67.80) 2.96
Eun BW 1 (2019) 62.40 (52.10, 72.70) 2.90
Oh CE (2018) 80.80 (76.30, 85.30) 2.96
Oh CE 1 (2018) 81.80 (73.20, 90.40) 2.92
Frey SE (2014) 67.60 (65.90, 69.20) 2.98
Subgroup, DL (I^2 = 96.7%, p = 0.000) 71.05 (65.21, 76.89) 38.15
Split
Cruz-Valdez (2018) 75.00 (65.90, 82.70) 2.92
Basu I (2021) 77.20 (71.30, 82.40) 2.95
zhang (2022) 78.15 (71.47, 80.71) 2.96
Diallo A (6-11 months) (2018) 73.10 (52.50, 88.40) 2.75
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NCT02255409 (2015) 56.40 (50.10, 62.60) 2.95
Vesikari T (2018) 73.67 (71.20, 76.10) 2.97
Subgroup, DL (I^2 = 91.6%, p = 0.000) 77.95 (70.69, 85.21) 23.35
Placebo
Beran J (2021) 2.10 (0.90, 4.30) 2.98
Mcbride WJH (2016) 1.00 (0.00, 4.00) 2.97
Subgroup, DL (I^2 = 0.0%, p = 0.411) 1.64 (0.34, 2.93) 5.95
Heterogeneity between groups: p = 0.000
Overall, DL (I^2 = 99.7%, p = 0.000) 73.91 (63.09, 84.73) 100.00
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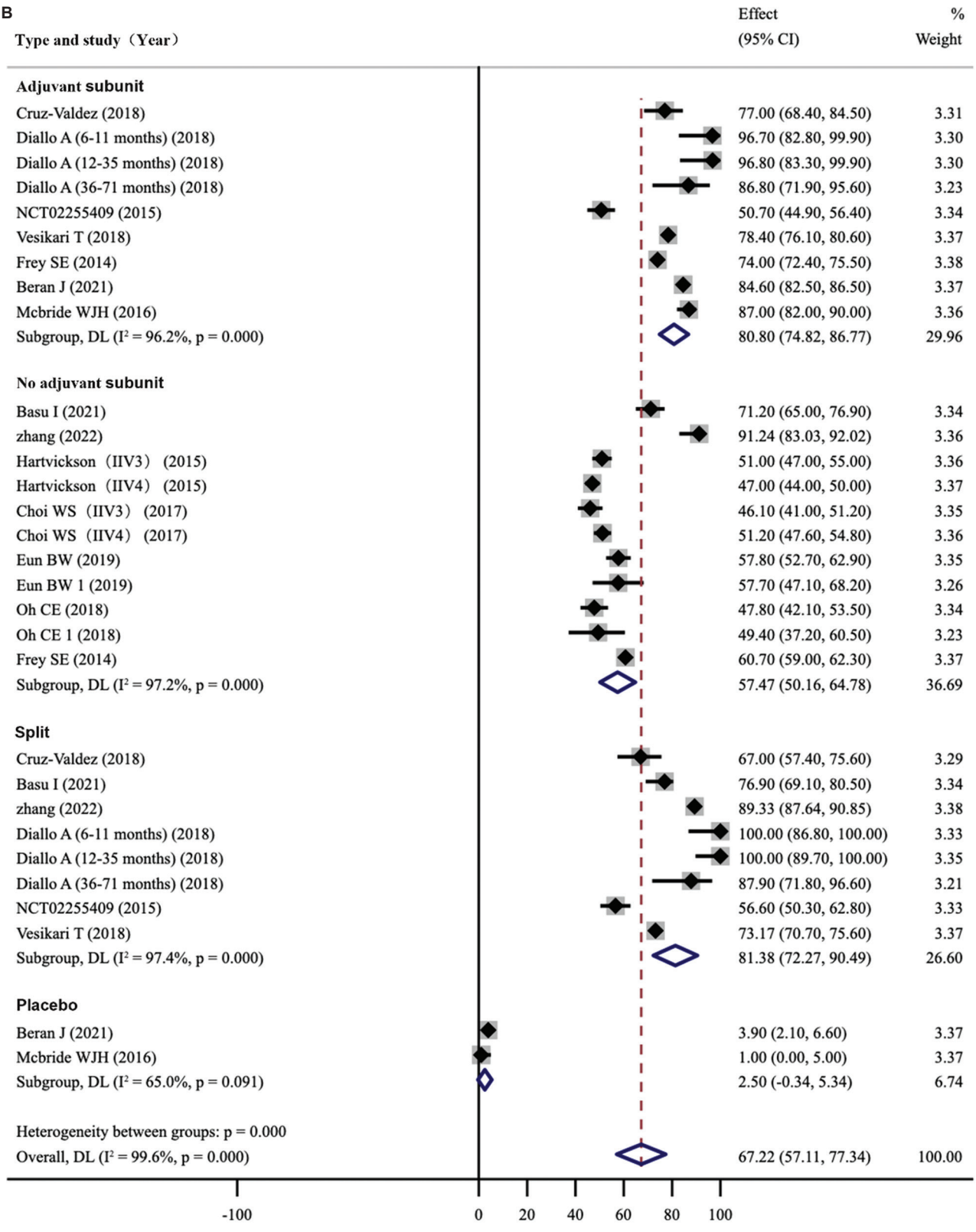
Figure 2. *(Continued)*



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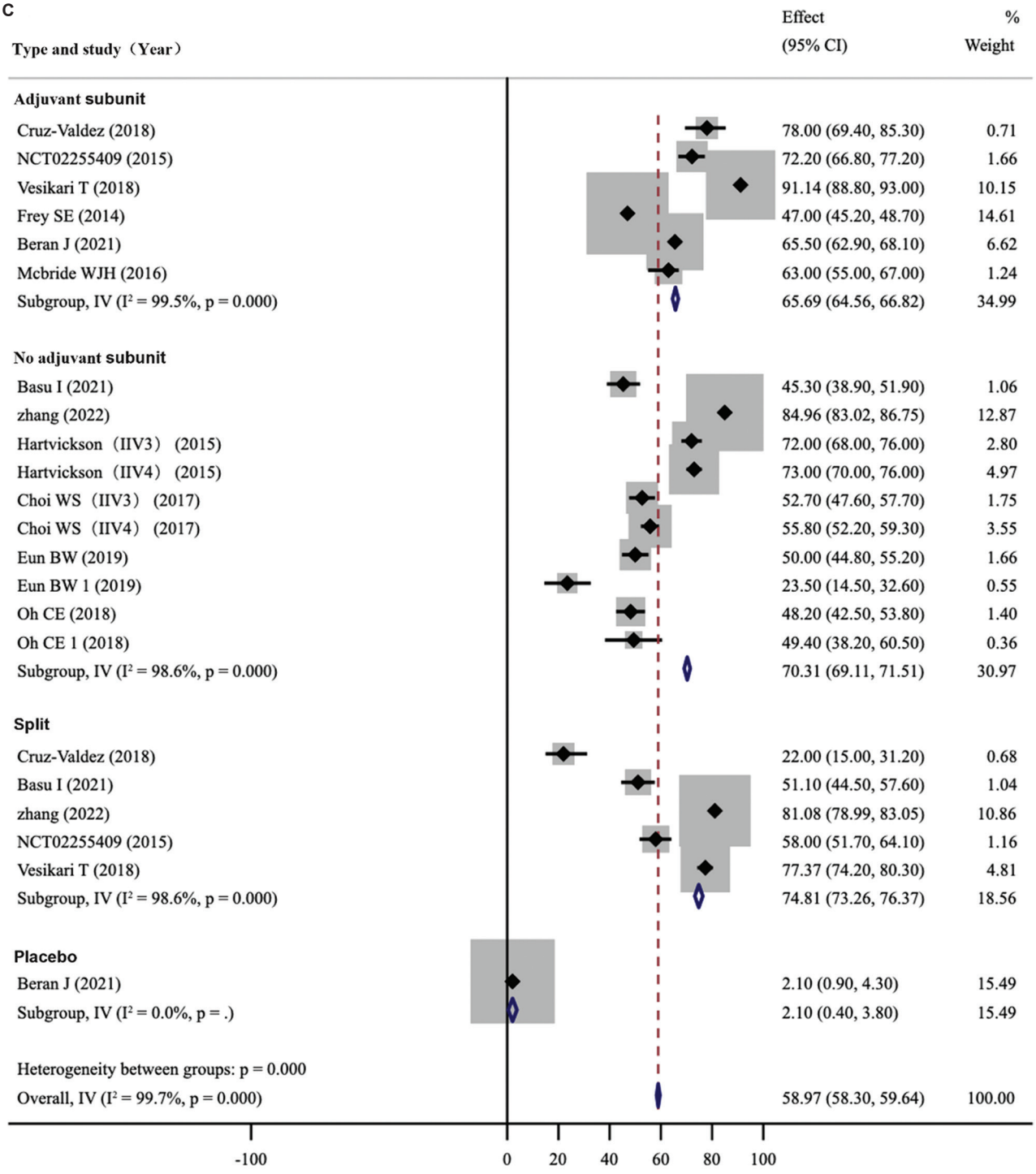

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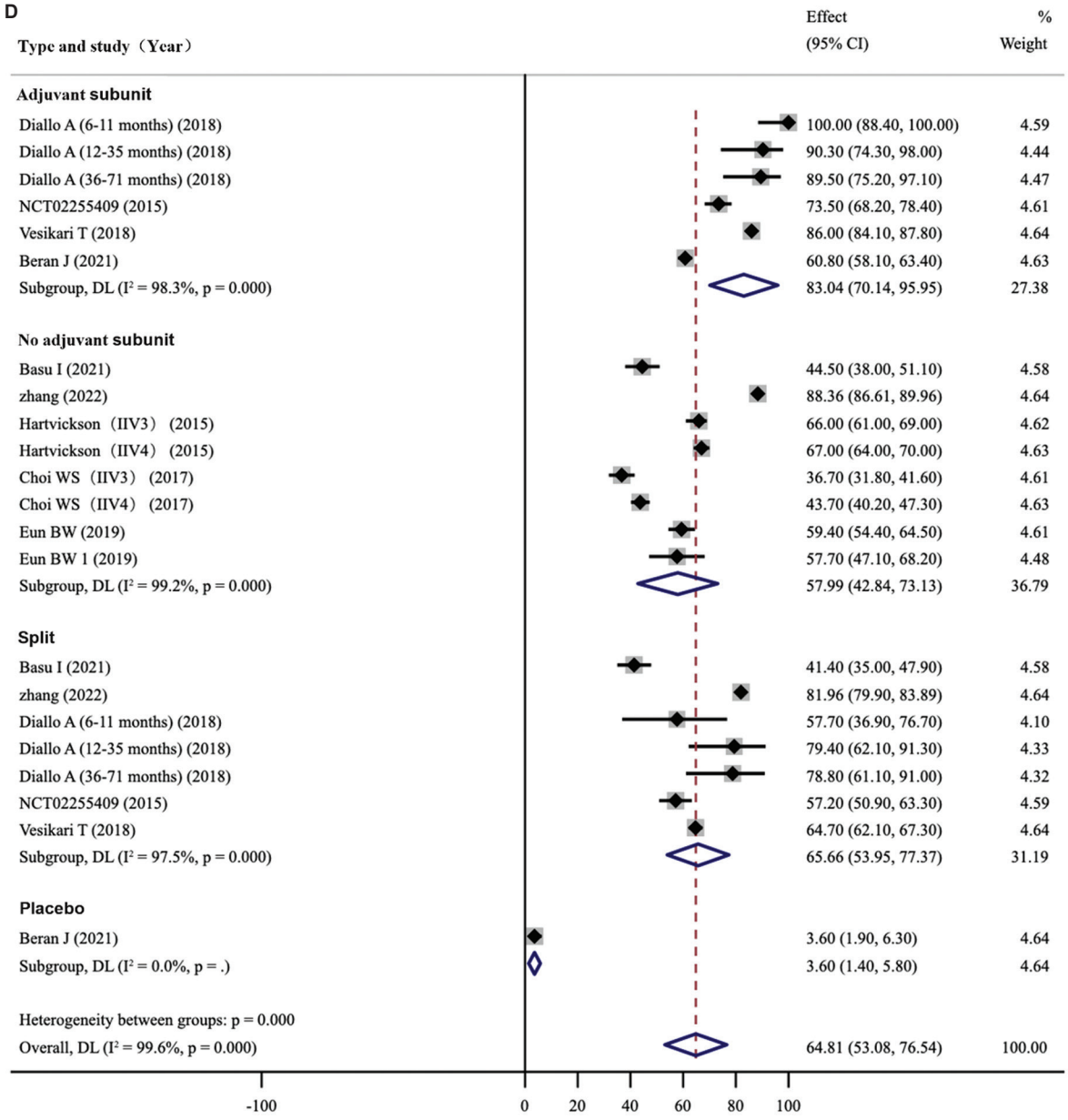
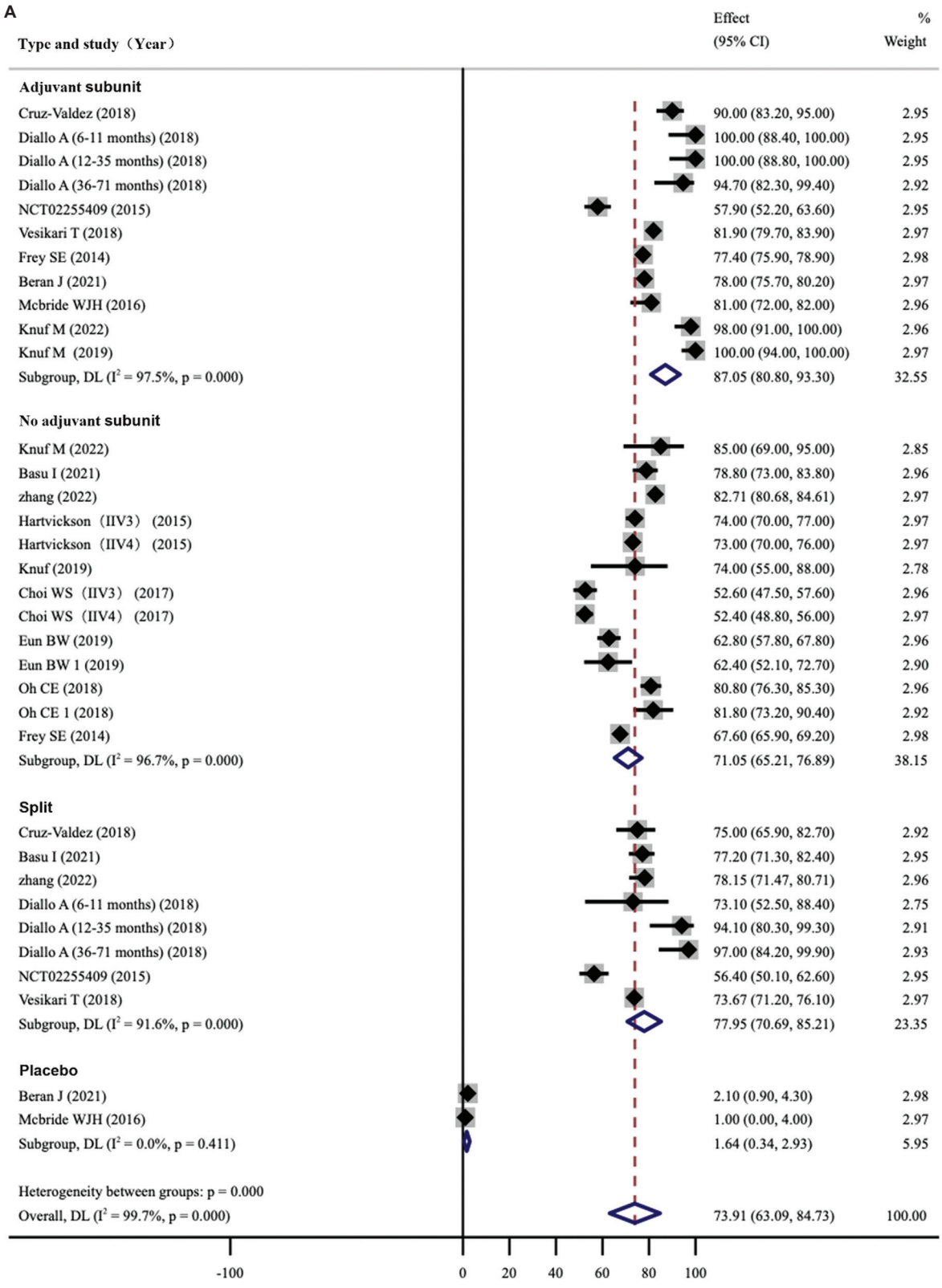


Figure 2. Forest plot of seroprotection rates for different influenza strains among different vaccine groups. Influenza strains: A/H1N1 strain (A); A/H3N2 strain (B); B/Victoria strain (C); B/Yamagata strain (D).
Abbreviations: DL: DerSimonian-Laird; IIV: Inactivated influenza vaccine; CI: Confidence interval.

Due to limitations in the number of clinical studies comparing non-adjuvanted subunit IV and split IV, we only compared the incidence of adverse events between individuals vaccinated with adjuvanted subunit IV and split IV. Table 3 features a forest plot displaying the occurrence rates of various adverse events across the two vaccine groups (adjuvanted subunit vaccine and split vaccine). Among the included studies reporting safety outcomes, the most frequent local side effects were induration/inflammation, ache, and redness. Systemic adverse events included chills, diarrhea, fatigue/somnolence, fever, headache, decreased appetite, and vomiting. Among these events, there were no significant differences in the incidence rates of induration/swelling, diarrhea, headache, and decreased appetite between the two groups, with low heterogeneity observed. However, for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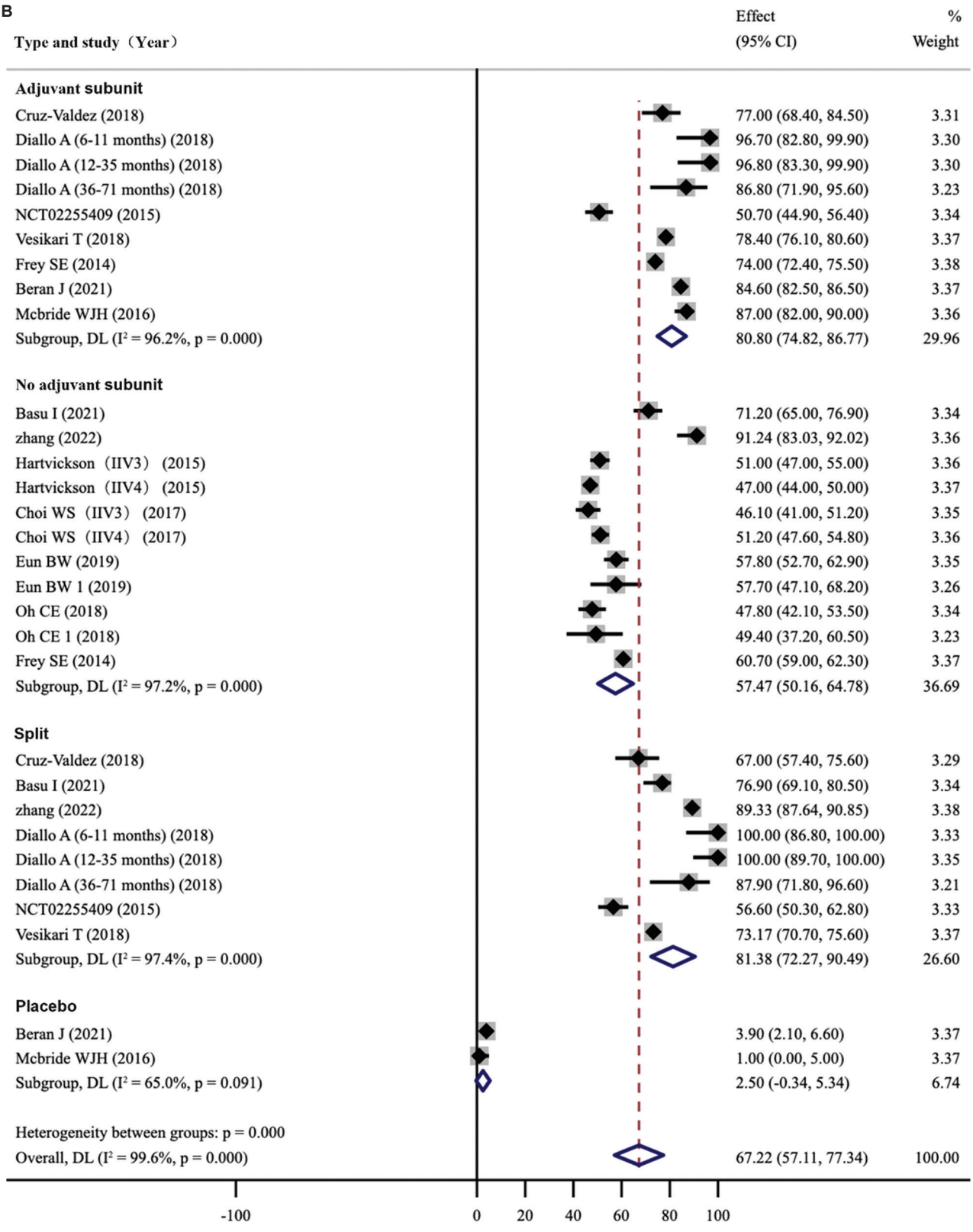

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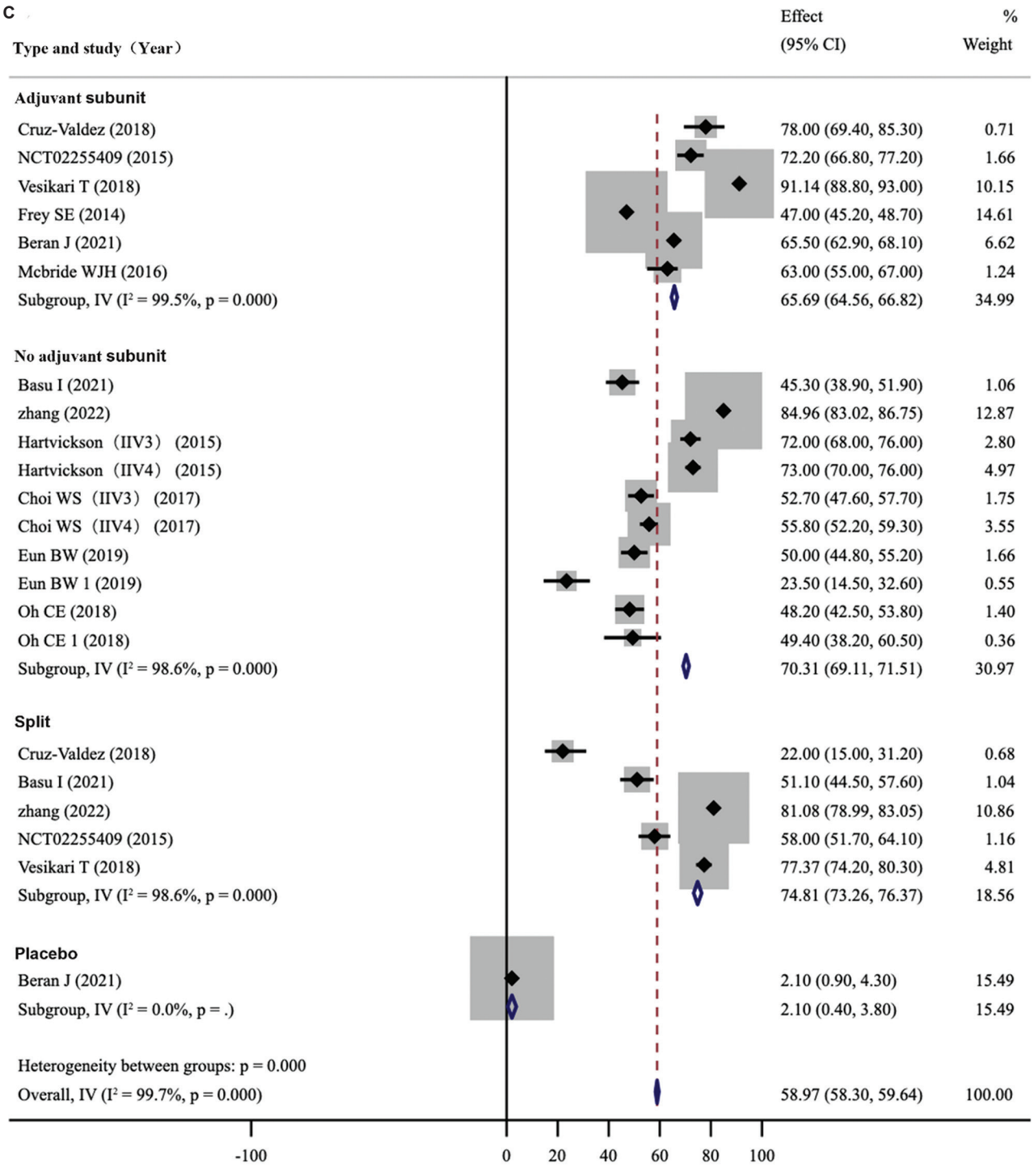

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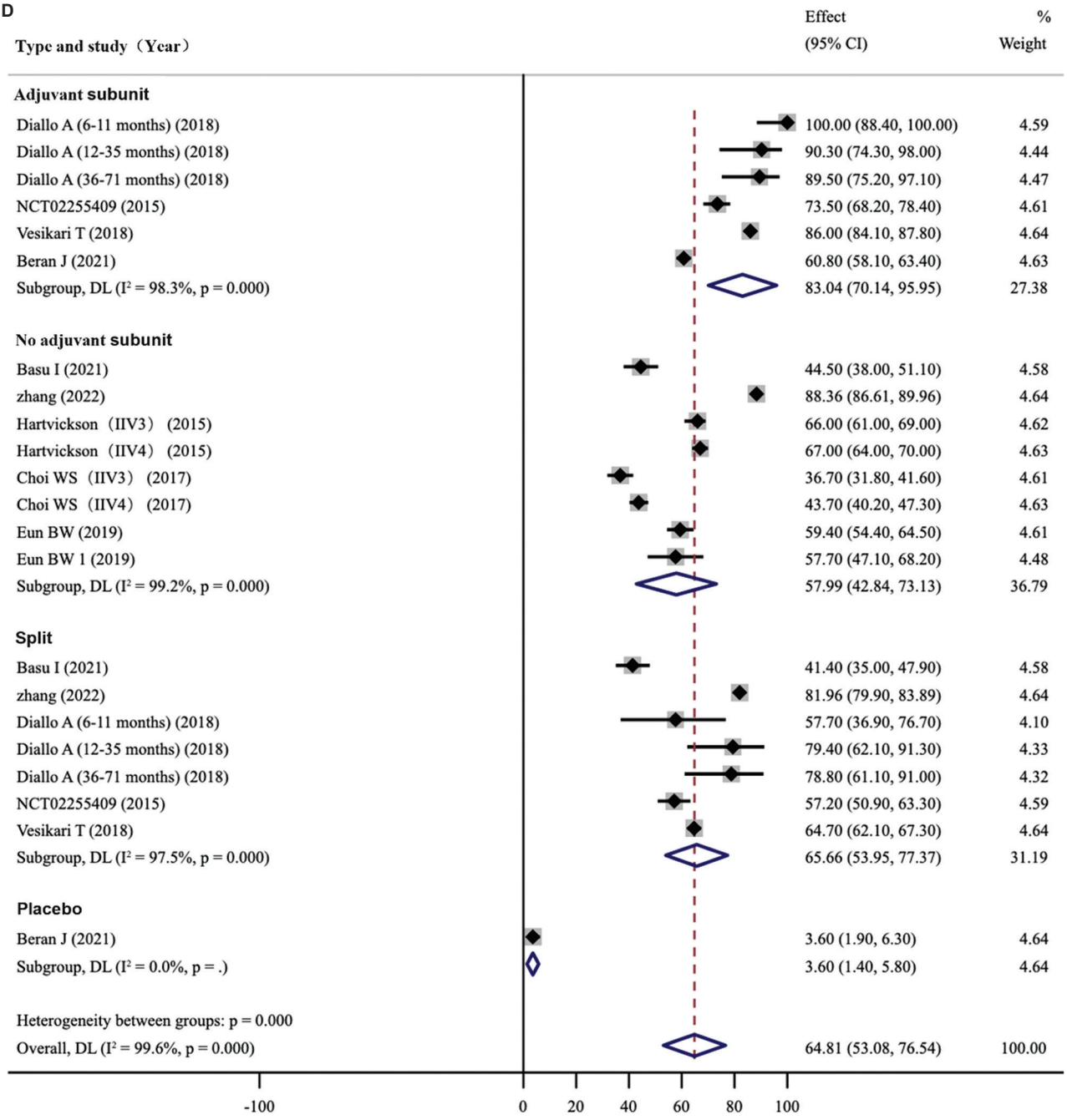

Figure 3. Forest plot of seroconversion rates for different influenza strains among different vaccine groups. Influenza strains: A/H1N1 strain (A); A/H3N2 strain (B); B/Victoria strain (C); B/Yamagata strain (D).
Abbreviations: DL: DerSimonian-Laird; IIV: Inactivated influenza vaccine; CI: Confidence interval.

other adverse reaction events (pain, redness, chills, fatigue/sleepiness, fever, and vomiting), the incidence rates were significantly lower in individuals vaccinated with split IV compared to those vaccinated with adjuvanted subunit IV.

4. Discussion

Influenza is one of the major diseases posing a serious threat to human health. Globally, seasonal influenza

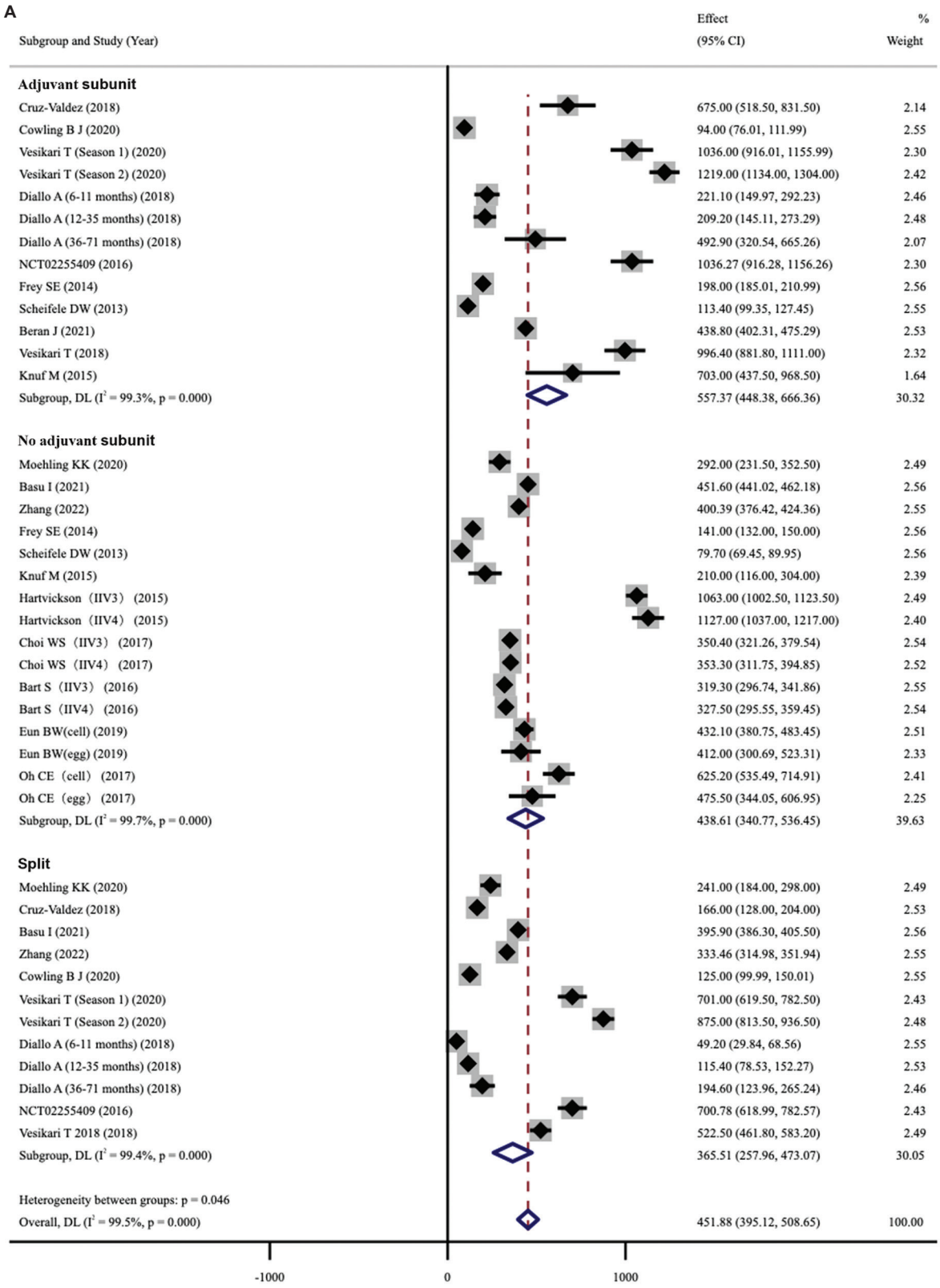
A
Subgroup and Study (Year)
Effect (95% CI)
% Weight
Adjuvant subunit
Cruz-Valdez (2018) 675.00 (518.50, 831.50) 2.14
Cowling B J (2020) 94.00 (76.01, 111.99) 2.55
Vesikari T (Season 1) (2020) 1036.00 (916.01, 1155.99) 2.30
Vesikari T (Season 2) (2020) 1219.00 (1134.00, 1304.00) 2.42
Diallo A (6-11 months) (2018) 221.10 (149.97, 292.23) 2.46
Diallo A (12-35 months) (2018) 209.20 (145.11, 273.29) 2.48
Diallo A (36-71 months) (2018) 492.90 (320.54, 665.26) 2.07
NCT02255409 (2016) 1036.27 (916.28, 1156.26) 2.30
Frey SE (2014) 198.00 (185.01, 210.99) 2.56
Scheifele DW (2013) 113.40 (99.35, 127.45) 2.55
Beran J (2021) 438.80 (402.31, 475.29) 2.53
Vesikari T (2018) 996.40 (881.80, 1111.00) 2.32
Knuf M (2015) 703.00 (437.50, 968.50) 1.64
Subgroup, DL (I^2 = 99.3%, p = 0.000) 557.37 (448.38, 666.36) 30.32
No adjuvant subunit
Moehling KK (2020) 292.00 (231.50, 352.50) 2.49
Basu I (2021) 451.60 (441.02, 462.18) 2.56
Zhang (2022) 400.39 (376.42, 424.36) 2.55
Frey SE (2014) 141.00 (132.00, 150.00) 2.56
Scheifele DW (2013) 79.70 (69.45, 89.95) 2.56
Knuf M (2015) 210.00 (116.00, 304.00) 2.39
Hartvickson (IIV3) (2015) 1063.00 (1002.50, 1123.50) 2.49
Hartvickson (IIV4) (2015) 1127.00 (1037.00, 1217.00) 2.40
Choi WS (IIV3) (2017) 350.40 (321.26, 379.54) 2.54
Choi WS (IIV4) (2017) 353.30 (311.75, 394.85) 2.52
Bart S (IIV3) (2016) 319.30 (296.74, 341.86) 2.55
Bart S (IIV4) (2016) 327.50 (295.55, 359.45) 2.54
Eun BW(cell) (2019) 432.10 (380.75, 483.45) 2.51
Eun BW(egg) (2019) 412.00 (300.69, 523.31) 2.33
Oh CE (cell) (2017) 625.20 (535.49, 714.91) 2.41
Oh CE (egg) (2017) 475.50 (344.05, 606.95) 2.25
Subgroup, DL (I^2 = 99.7%, p = 0.000) 438.61 (340.77, 536.45) 39.63
Split
Moehling KK (2020) 241.00 (184.00, 298.00) 2.49
Cruz-Valdez (2018) 166.00 (128.00, 204.00) 2.53
Basu I (2021) 395.90 (386.30, 405.50) 2.56
Zhang (2022) 333.46 (314.98, 351.94) 2.55
Cowling B J (2020) 125.00 (99.99, 150.01) 2.55
Vesikari T (Season 1) (2020) 701.00 (619.50, 782.50) 2.43
Vesikari T (Season 2) (2020) 875.00 (813.50, 936.50) 2.48
Diallo A (6-11 months) (2018) 49.20 (29.84, 68.56) 2.55
Diallo A (12-35 months) (2018) 115.40 (78.53, 152.27) 2.53
Diallo A (36-71 months) (2018) 194.60 (123.96, 265.24) 2.46
NCT02255409 (2016) 700.78 (618.99, 782.57) 2.43
Vesikari T 2018 (2018) 522.50 (461.80, 583.20) 2.49
Subgroup, DL (I^2 = 99.4%, p = 0.000) 365.51 (257.96, 473.07) 30.05
Heterogeneity between groups: p = 0.046
Overall, DL (I^2 = 99.5%, p = 0.000) 451.88 (395.12, 508.65) 100.00
-1000
0
1000

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Figure 4. *(Continued)*

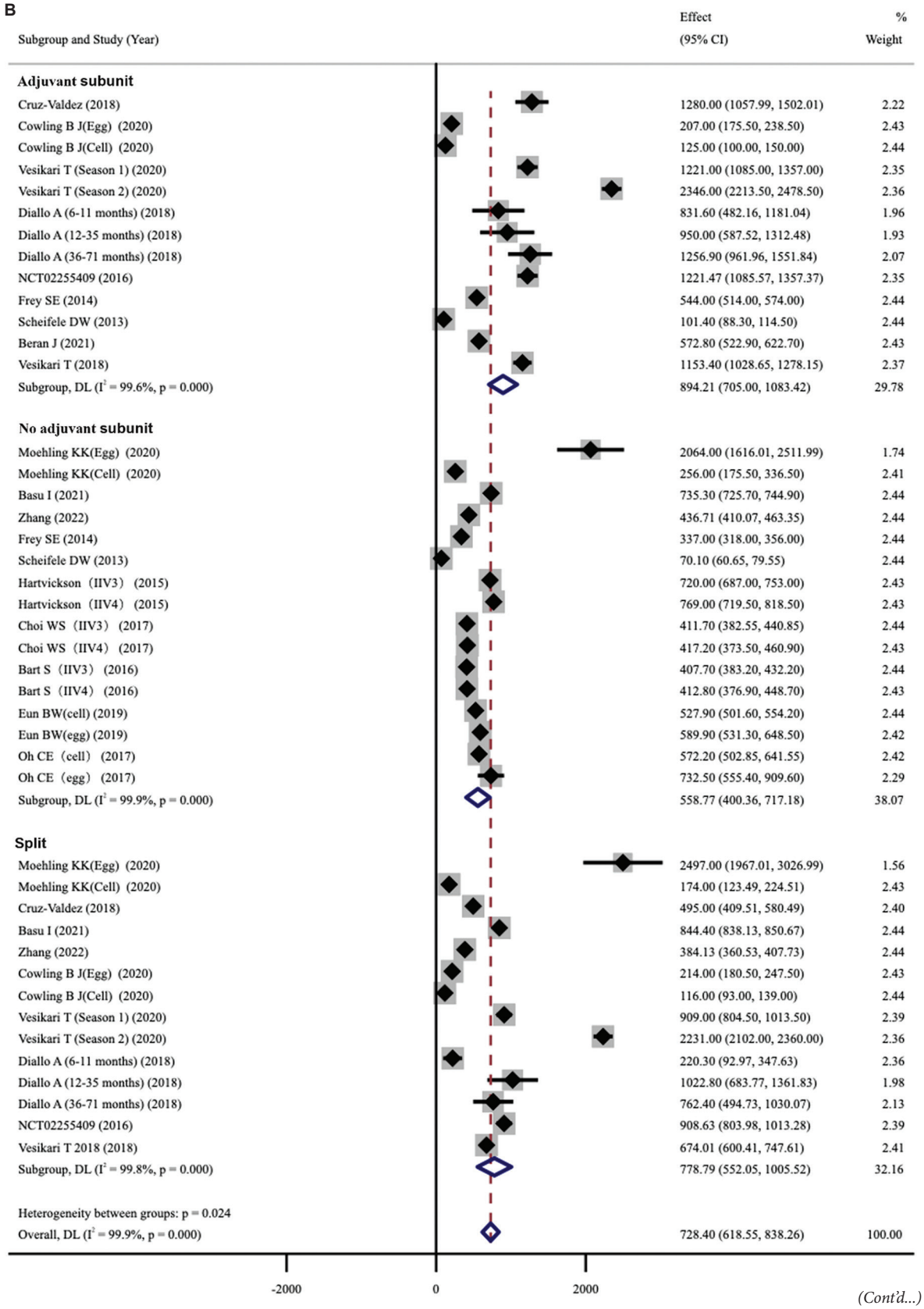

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Figure 4. *(Continued)*

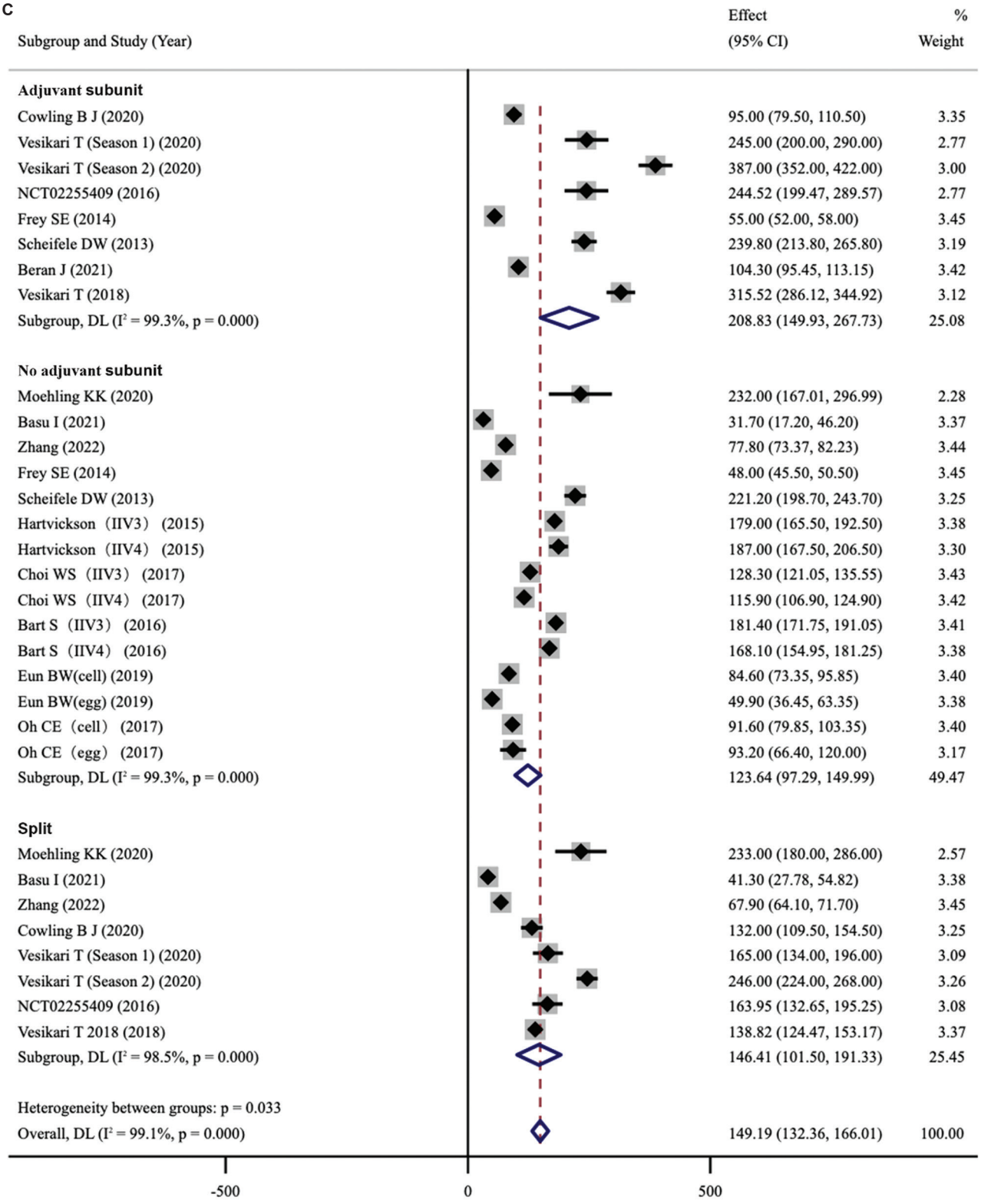

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Figure 4. *(Continued)*

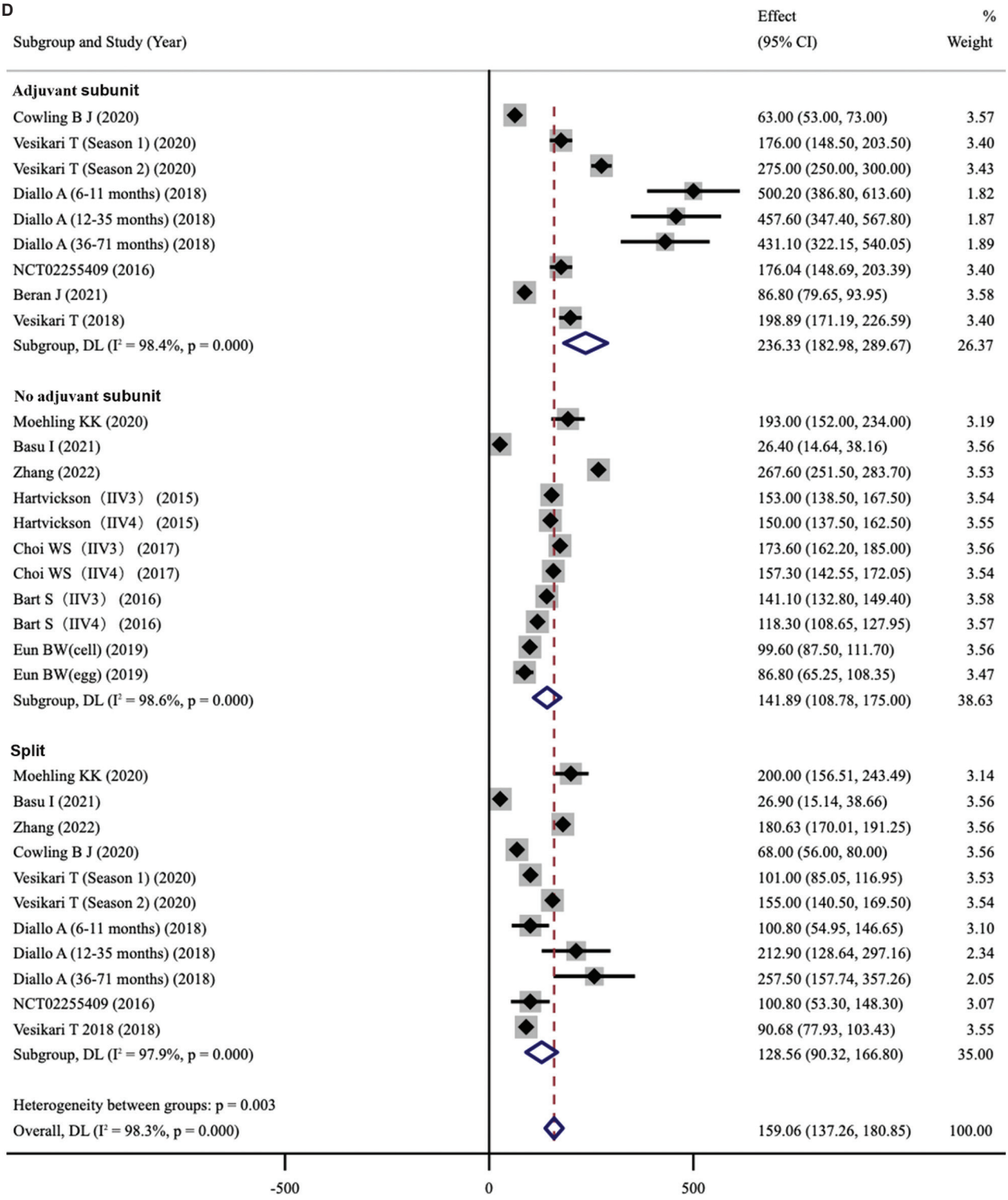

Figure 4. Forest plots of GMTs for different influenza strains among different vaccine groups. Influenza strains: A/H1N1 strain (A); A/H3N2 strain (B); B/Victoria strain (C); B/Yamagata strain (D).
Abbreviations: DL: DerSimonian-Laird; GMT: Geometric mean titer; IIV: Inactivated influenza vaccine; CI: Confidence interval.

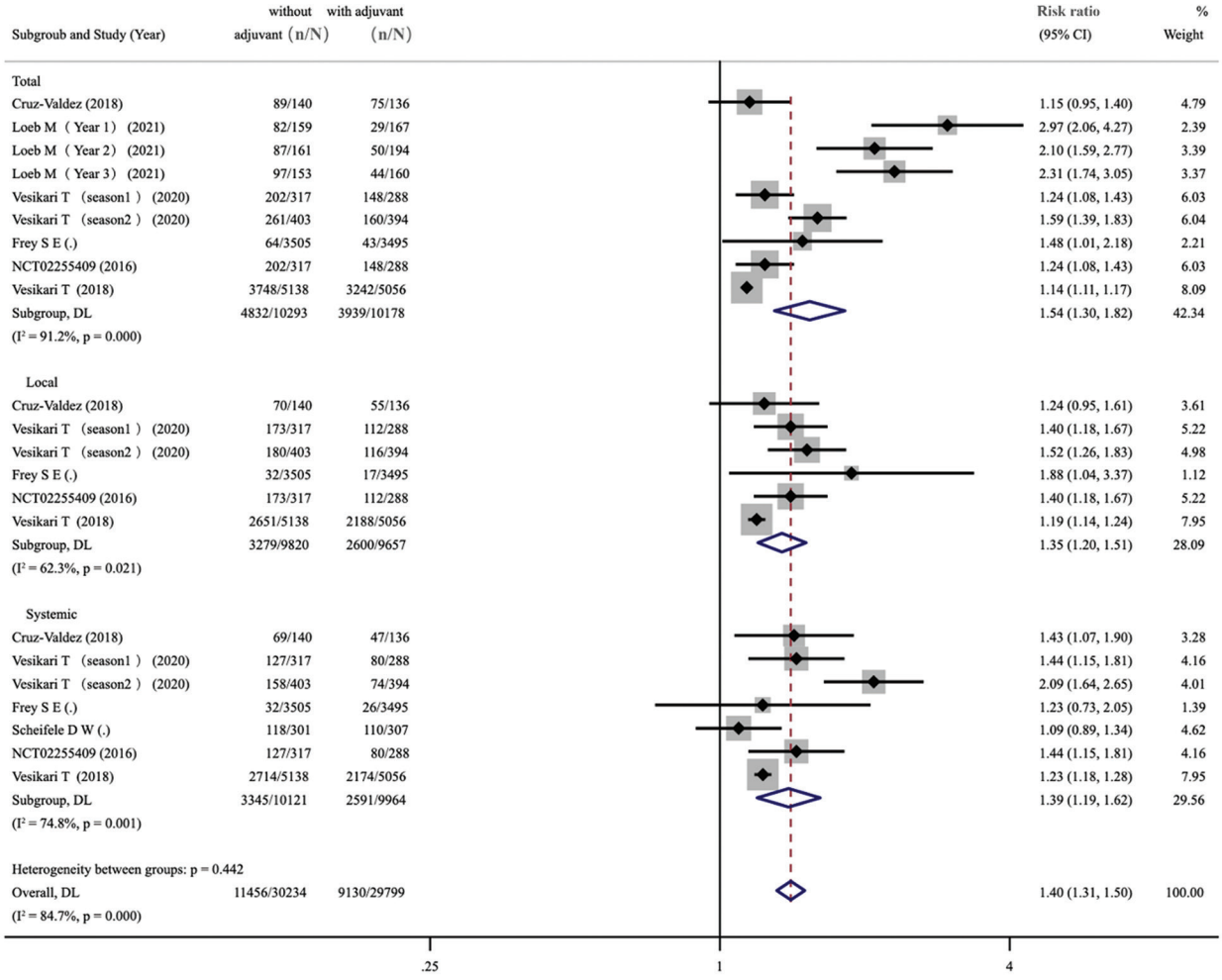

Figure 5. Forest plot of adverse event rates between the two vaccine groups (with or without adjuvant)
Abbreviations: DL: DerSimonian-Laird; GMT: Geometric mean titer; IIV: Inactivated influenza vaccine; CI: Confidence interval.

Table 2. Forest plot data of adverse events rates between the two vaccine groups (subunit vaccines vs. split vaccines)

Group	Adverse events	No. of clinical result	Analysis model	RR	95% CI (%)	I^2 (%)	*p*
Non-adjuvanted subunit vaccines versus split vaccines	Total adverse events	2	FE	1.03	0.89 – 1.19	0.0	0.693
	Systemic adverse events	1	FE	1.11	0.88 – 1.39	0.0	0.658
	Local adverse events	1	FE	1.01	0.82 – 1.24	0.0	0.393
	Overall	2	FE	1.04	0.94 – 1.16	0.0	0.917
Adjuvanted subunit vaccines versus split vaccines	Total adverse events	10	RE	1.54	1.29 – 1.84	91.1	0.000
	Systemic adverse events	6	RE	1.33	1.19 – 1.49	64.4	0.024
	Local adverse events	6	RE	1.48	1.22 – 1.80	81.9	0.000
	Overall	10	RE	1.42	1.32 – 1.52	86.5	0.000

Abbreviations: CI: Confidence interval; FE: Fixed-effects model; RE: Random-effects model; RR: Risk ratio.

accounts for approximately one billion cases each year, of which 3 – 5 million are severe. These epidemics result in numerous infections and can cause severe health issues, particularly among populations at higher risk due to age or underlying medical conditions.[14,15] Vaccination against influenza is the most effective way to prevent the flu and its associated health complications.[16] This study aimed to offer a general review and meta-analysis of the

Table 3. Forest plot data of the incidence of various adverse events between the two vaccine groups (subunit vaccines vs. split vaccines)

Adverse events	No. of clinical result	Analysis model	RR	95% CI (%)	I^2 (%)	*p*
Local adverse events						
Induration/swelling	7	FE	1.44	1.31 – 1.58	30.7	0.193
Pain	6	RE	1.67	1.29 – 2.17	76.1	0.001
Redness	7	RE	1.48	1.16 – 1.89	64.6	0.010
Systemic adverse events						
Chills	7	RE	2.98	1.63 – 5.45	70.7	0.002
Diarrhea	8	FE	1.06	0.96 – 1.16	24.8	0.231
Fatigue/sleepiness	6	RE	1.42	1.04 – 1.93	79.7	0.001
Fever	8	RE	3.09	1.89 – 5.05	89.8	0.000
Headache	4	RE	3.14	1.34 – 7.38	47.3	0.128
Loss of appetite	7	FE	1.86	1.50 – 2.31	35.1	0.160
Vomiting	8	RE	2.03	1.27 – 3.23	68.8	0.002

Abbreviations: CI: Confidence interval; FE: Fixed-effects model; RE: Random-effects model; RR: Risk ratio.

current status of IV administration, comparative studies of adjuvanted subunit IVs and split vaccines, and relevant clinical research on subunit IVs. The study objective is to understand the differences in immunogenicity and safety of non-adjuvanted subunit IVs, adjuvanted subunit IVs, and split vaccines. This research intends to offer insights and directions for future studies on IVs and establish theoretical foundations for IV prophylactic regimens.

The research findings indicated that within 20 – 30 days after receiving the adjuvanted subunit IV, non-adjuvanted subunit IV, or split IV, subjects demonstrated robust immunogenicity against influenza A/H1N1 and influenza A/H3N2 strains. However, the overall immunogenicity against influenza B strains BY and BV was comparatively lower. For the influenza A strains, the adjuvanted subunit IV displayed higher seroconversion rates and GMTs compared to the non-adjuvanted subunit IV and split IV. These findings align with other studies,[14-16] suggesting superior immunogenicity of the adjuvanted subunit IV. Nevertheless, in terms of seroprotection rates, the non-adjuvanted subunit IV demonstrated similar protection rates to the adjuvanted subunit IV for all four influenza strains. This implies that, for most individuals, the non-adjuvanted subunit IV can offer sufficient protection. However, the study results are limited by the single arm trial design, warranting further controlled experiments to validate these findings.

Research on the repeated administration and long-term effectiveness of IVs is worthwhile,[17] but this aspect was not systematically analyzed in this study due to limited literature. Our research group previously published results suggesting that a single dose of the 2009 pandemic influenza A/H1N1 split virus vaccine, containing different doses (15, 30, and 45 μg) of HA, could induce a protective immune response lasting at least 6 months in adults.[18] However, by day 360, none of the dosage cohorts achieved seroprotection rates exceeding 70%. In addition, Vesikari *et al.*[19] found that the vaccine recipients maintained high immune potency after receiving repeated doses of adjuvanted quadrivalent IV 6 months apart. Kuff *et al.*[20] observed persistent antibody levels 1 year after administering adjuvanted monovalent influenza A/H1N1 vaccine in the 3 – 17 age group. In a separate study, Kuff *et al.*[21] further validated these findings, with 95–100% of subjects receiving adjuvanted monovalent H1N1 IV maintaining HI titers ≥1:40 1 year after immunization; the effectiveness of the vaccine may diminish with repeated administration. In 2023, Jones-Gray *et al.*[22] performed a systematic review that reported similar outcomes, indicating decreased vaccine effectiveness with repeated administration, but two consecutive years of vaccination provided better protection compared to no vaccination. The immunogenicity of consecutive seasons of cell culture-based inactivated IV (IIV) is similar to consecutive seasons of egg-based IIV. The difference in HA antigen content plays a role in the immunogenicity of consecutive seasonal influenza vaccinations. Trombetta *et al.*[23] conducted a study on adult healthcare workers and found that consecutive vaccination with recombinant-HA IV (RIV; with each component containing 45 μg of HA) in two sequential seasons or the latter year displayed greater immunogenicity compared to sequential vaccination with egg-based IIV (each component containing 15 μg of HA) for three out of four components (A/H1N1, BV, and BY) of the quadrivalent vaccine. Only a few studies have touched on the effectiveness of repeated administration, but none have provided definitive conclusions.[24] In general, research on repeated administration and the long-term effectiveness

of IVs is still ongoing, and current research results are not yet conclusive.

The incidence of adverse events is a crucial indicator for assessing the safety of IVs.[24] Subunit IVs, which enhance the purity of effective antigens compared to split IVs, are expected to provide increased safety.[9,25] However, our systematic review revealed that the incidence of adverse reaction events in most adjuvanted subunit IVs was higher than that in split IVs. This may be due to the additional side effects associated with adjuvants, which are consistent with previous studies.[26] We further compared the impact of adjuvant addition on the safety of subunit IVs and bolstered this viewpoint. The incidence of adverse events from adjuvanted subunit IVs was significantly higher than that from non-adjuvanted subunit IVs. However, most studies did not extensively address the severity of adverse events, necessitating further research to confirm this point. In some studies of non-adjuvanted subunit IVs, we found that they may offer greater safety. However, relative to adjuvanted subunit IVs, the immunogenicity of non-adjuvanted subunit IVs may be slightly reduced, although still comparable to that of split IVs. Therefore, for the majority of the population, non-adjuvanted subunit IVs should be prioritized when receiving the IV to minimize the risk of adverse reaction events. Adjuvanted subunit IVs are recommended for populations with lower immune capability. Nevertheless, when formulating vaccination plans, individual medical conditions and recommendations should still be considered comprehensively.

This is the first meta-analysis to compare the immunogenicity and safety of non-adjuvanted subunit IVs, adjuvanted subunit IVs, and split IVs. The study conducted a comprehensive analysis by systematically summarizing all clinical research data comparing subunit IVs with split IVs and adjuvanted subunit IVs, providing a more comprehensive and intuitive display of the immunogenicity and safety of IVs. This search was conducted on the most relevant databases, and appropriate measures were taken to select studies and extract data to prevent potential errors, thus minimizing selection bias. In addition, the included studies exhibited minimal risk of bias, allowing us to consider the evaluation results to be robust. This meta-analysis is based on an extensive search strategy, but the primary literature has certain limitations. The aim of RCTs is to determine the immunogenicity and safety of IVs and not to compare the effectiveness of vaccination after exposure.[17] Furthermore, there is still a lack of research comparing the immunogenicity and safety of non-adjuvanted subunit IVs with adjuvanted IVs, as well as non-adjuvanted subunit IVs with split IVs. Variations in adjuvant dosage have also led to wide confidence intervals. The sustained presence of antibodies over a long-term serves as a crucial marker for immunogenicity; however, investigation in this area is still remarkably limited in subsequent studies of GMTs and seroconversion. In addition, the seasonal impact of influenza strains is another significant factor. Despite the comprehensive nature of our search protocol and the use of extensive search terms, some relevant studies may have been overlooked.

5. Conclusion

This study indicated that adjuvanted subunit IVs, non-adjuvanted IVs, and split IVs demonstrated strong performance in terms of immunogenicity. The use of adjuvanted subunit IVs can enhance immunogenicity, but the addition of adjuvants may increase the occurrence of adverse events. In contrast, split IVs and non-adjuvanted subunit IVs exhibit comparable levels of protective capacity and immunogenicity. Therefore, for the majority of the population, it is recommended to use non-adjuvanted subunit IVs to ensure better safety. For populations with weaker immune responses, the use of adjuvanted IVs is recommended to provide enhanced immunogenicity. However, it is essential to highlight that the quantity and quality of the included studies are limited at present; further high-quality research is necessary to validate these results and ensure their accuracy and reliability. Furthermore, due to the high variability of influenza virus strains, there is a need for more research on the effects of repeated influenza vaccination and the long-term protective efficacy of IVs. This research direction is crucial for improving vaccination strategies and further reducing the spread of influenza.

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Conflict of interest

All authors declare that they have no conflicts of interest.

Author contributions

Conceptualization: Ze Chen
Formal analysis: Lei Wang
Investigation: Lei Wang, Dan Li, Hongbo Zhang, Youcai An, Xinyue Zhang
Methodology: Lei Wang, Hongbo Zhang, Youcai An, Xinyue Zhang
Writing – original draft: Lei Wang, Ze Chen

Writing – review & editing: Lei Wang, Hongbo Zhang, Ze Chen

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author on reasonable request.

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ACCSCIENCE PUBLISHING

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REVIEW ARTICLE

Greek poplar-type propolis as an adjunct therapy in hospitalized COVID-19 adults: A randomized controlled trial protocol

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Abstract

Background: Despite the rapid development and distribution of COVID-19 vaccines, the pandemic continues to challenge global health systems. With vaccine inequity and hesitancy, especially in low-income populations and specific demographic cohorts, alternative therapeutic strategies to mitigate COVID-19 symptoms and reduce viral clearance time remain vital. Propolis, a natural bee product with immunomodulatory and antiviral properties, has demonstrated efficacy against other viral pathogens, suggesting potential as an adjunctive therapy for COVID-19. **Objectives**: This study protocol outlines a randomized, triple-blind, placebo-controlled clinical trial to assess the efficacy of a Greek propolis hydroalcoholic extract as an adjunct to standard care in hospitalized COVID-19 patients. The primary objectives are to evaluate the extract's impact on viral clearance time and hospitalization duration, with secondary objectives examining body temperature, cough severity, quality of life, and safety. **Methods**: A total of 441 severe acute respiratory syndrome coronavirus 2-positive adult patients will be enrolled and stratified by age and vaccination status. Participants will be randomly assigned to one of three arms: (i) propolis extract, (ii) placebo, or (iii) control (standard care only). Primary outcomes include time to negative reverse transcription polymerase chain reaction tests and hospital discharge. Secondary measures involve cough severity and quality-of-life assessments through Visual Analog Scale and Leicester Cough Questionnaire scores, fever duration and resolution patterns, and safety through adverse events and mortality tracking. Statistical analysis will include Kaplan–Meier survival curves, Cox regression for confounders, and analysis of variance for quality-of-life scores. **Conclusion**: This study aims to validate the therapeutic potential of propolis as a natural, accessible adjunctive treatment for COVID-19. Findings may provide critical evidence supporting propolis in symptom relief, viral clearance, and healthcare burden reduction in resource-limited settings. **Relevance for patients:** Participants in the intervention arm may experience improved clinical outcomes, such as faster recovery and symptom alleviation, while all patients will continue to receive standard care in alignment with current clinical protocols.

Keywords: SARS-CoV-2; COVID-19; Propolis; Adjunct therapy; Nutraceuticals; Randomized controlled trial; Immune modulation

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1. Introduction

The COVID-19 pandemic continues to challenge global healthcare systems, despite the rapid rollout of vaccines and public health interventions. Vaccine inequity remains a major obstacle, particularly in low- and middle-income countries, where a significant proportion of the population still lacks access to vaccination.[1,2] In addition, vaccine hesitancy has been documented among specific demographic cohorts, including healthcare workers, driven by concerns over safety, misinformation, and distrust in the pharmaceutical industry.[3-5]

These gaps underscore the urgent need for safe, effective, and affordable adjunctive therapies to complement standard care, particularly those that are accessible in resource-limited settings. Propolis, a complex resinous product collected by bees from plant exudates, has been used medicinally for its antimicrobial, anti-inflammatory, and immunomodulatory properties.[6,7] Contemporary *in vitro* and *in silico* studies have demonstrated that flavonoids and other polyphenolic constituents of propolis can inhibit viral replication and modulate immune pathways.[8-11] Moreover, early-stage clinical investigations suggest potential benefits in symptom reduction and viral clearance in COVID-19; however, methodological limitations restrict the generalizability of these findings.[12-15]

The chemical composition and therapeutic profile of propolis vary by geography and botanical source. Greek poplar-type propolis, rich in polyphenols,[16] may offer targeted antiviral effects relevant to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, no robust placebo-controlled clinical trials have yet evaluated its efficacy in COVID-19 treatment.

This protocol describes a randomized, triple-blind, placebo-controlled three-arm clinical trial investigating the therapeutic potential of a Greek hydroalcoholic propolis extract, formulated into alcohol-free oral capsules, as an adjunct to standard care, compared against both a placebo adjunct and standard care alone. The primary outcomes are time to viral clearance and duration of hospitalization, with secondary endpoints examining symptom severity, quality of life (QoL), and safety.

2. Background and rationale of the study

Amid persistent global disparities in vaccine distribution and uptake, there has been growing interest in accessible, safe adjuncts to conventional COVID-19 care. During the pandemic, global demand for nutraceuticals and botanical supplements surged, driven by perceptions of immune-enhancing effects and widespread mistrust toward pharmaceutical interventions.[17-19] Despite limited clinical validation, such products have been widely adopted, particularly in low-resource settings and among vaccine-hesitant populations.[20] Reflecting this trend, the World Health Organization (WHO) has highlighted the role of traditional medicine in public health, noting that approximately 80% of the global population uses natural therapies.[21] In this context, natural agents with historical use and emerging antiviral evidence, such as propolis, warrant further scientific investigation.

Propolis is a resinous substance produced by bees from plant exudates, widely used in traditional medicine for its antimicrobial and wound-healing properties.[22,23] Its composition varies depending on geographic origin and botanical source, influencing its bioactivity. The poplar-type propolis found in Greece is rich in flavonoids and phenolic compounds such as pinocembrin, galangin, chrysin, and caffeic acid phenethyl ester, which have been associated with antiviral, anti-inflammatory, and immunomodulatory effects.[8,16]

Various systematic reviews and pre-clinical studies support the potential of propolis as a candidate for COVID-19 adjunctive therapy. A 2022 systematic review by Dilokthornsakul *et al.*[11] summarized nine *in silico* studies showing strong binding affinities between key flavonoids in propolis, such as caffeic acid phenethyl ester, chrysin, luteolin, and rutin, and SARS-CoV-2 targets, including the angiotensin-converting enzyme 2 receptor, main protease, papain-like protease, and RNA-dependent RNA polymerase. These constituents were found to interact with viral entry and replication pathways, often demonstrating comparable or superior docking scores to repurposed antiviral drugs.

Complementary *in vitro* investigations have further validated these findings. Sberna *et al.*[24] reported that Eurasian poplar-type propolis extract inhibited SARS-CoV-2 replication in Vero E6 cells, reduced cytopathic effects, and modulated proinflammatory cytokines. Other *in vitro* studies with flavonoids commonly found in propolis, such as rutin, hypericin, and cyanidin-3-O-glucoside, have demonstrated concentration-dependent inhibition of SARS-CoV-2 proteases.[25,26] While variability in composition presents a challenge, the Greek variant, classified as poplar-type, shares a similar phytochemical profile.

Clinical data, though still limited, are emerging. A randomized controlled trial by Silveira *et al.*[14] using Brazilian green propolis (EPP-AF®) showed reduced hospital stay in COVID-19 patients. In addition, a volunteer-based study[15] and two case studies[12,13] have described symptom improvement, faster recovery, and possible prophylactic effects following propolis

supplementation. However, these studies differ in extract standardization, dosage, and methodological quality, underscoring the need for rigorously designed clinical trials using reproducible formulations.

Historically, propolis has been employed in folk medicine for wound healing, respiratory infections, and inflammation, with documented use in Ancient Egypt, Greece, and China, lending support to its recognized safety profile.[27,28] In parallel, studies today have provided evidence that propolis exhibits numerous antipathogenic and immunoregulatory properties.[29-33]

Given its long-standing use, favorable safety profile, and emerging antiviral evidence, propolis presents as a compelling candidate for integrative approaches to COVID-19 management. The extract proposed in this study is a standardized hydroalcoholic solution derived from Greek poplar-type propolis, produced under Good Manufacturing Practice conditions. Its chemical profile has been characterized in previous pharmacognostic studies and aligns with European Pharmacopeia guidelines for phenolic content.

This trial is designed to evaluate its potential as an adjunctive therapy to standard care in hospitalized COVID-19 patients, focusing on time to viral clearance, length of hospitalization, symptom severity, and safety outcomes.

3. Objectives

The primary objective is to determine whether the Greek poplar-type propolis extract can accelerate SARS-CoV-2 viral clearance and reduce hospitalization duration when used as an adjunct to standard care. Secondary objectives include evaluating changes in body temperature, cough severity and duration, cough-specific QoL, safety, and mortality.

4. Study design

This will be a randomized, triple-blind, placebo-controlled, three-arm clinical trial conducted at one or more tertiary-care hospitals.

A total of 441 hospitalized adults aged 18 – 85 with COVID-19 (polymerase chain reaction [PCR] test-confirmed) will be randomly assigned to receive either (i) standard care plus propolis extract, (ii) standard care plus placebo, or (iii) standard care alone. Each group will include 147 participants.

Randomization will be stratified by age and vaccination status using computer-generated sequences. Investigators, healthcare providers, and patients will be blinded to treatment allocation. Ethical approval will be sought before initiation, and informed consent will be obtained from all participants.

5. Methodology

Eligible participants will be adults aged between 18 and 85 years, with a confirmed SARS-CoV-2 infection as verified through negative reverse transcription PCR (RT-PCR) testing. Only individuals who are able to consume oral medications will be considered. Furthermore, participation will require the provision of written informed consent.

Individuals will be excluded from the study if they present with severe hepatic or renal impairment, if they have known hypersensitivity to propolis or alcohol-based formulations, or if they are currently enrolled in another clinical trial. Additional exclusion criteria include pregnancy or lactation, due to the absence of sufficient safety data regarding the use of propolis in these populations.

Participants in the intervention group will receive 800 mg/day of standardized, alcohol-free propolis capsules for 10 days. This dosage was selected based on similar human studies in which doses ranging from 375 mg to 800 mg/day were found to be safe and well-tolerated.[14,34,35] In particular, the study by Silveira *et al.*[14] administered 800 mg/day as an adjunct therapy in hospitalized COVID-19 patients without observing adverse effects, supporting the rationale for adopting the same dosage.

The extract will be prepared using ultrasound-assisted hydroalcoholic extraction and then spray-dried into a tasteless powder. The placebo arm will receive placebo capsules identical in appearance, containing inert starch. Standard care will be provided in all arms.

6. Outcome measures

6.1. Primary study outcomes

The primary outcome of this study will assess whether the intervention accelerates SARS-CoV-2 viral clearance and reduces hospitalization duration, compared to placebo and standard care. Viral shedding will be evaluated through RT-PCR tests using nasopharyngeal swabs, conducted on days 1 (baseline), 5, 7, 10, 14, 15, 16, and 17. Once a negative result is obtained, no further PCR tests will be performed. These time points were selected based on prior meta-analyses indicating a SARS-CoV-2 RNA shedding duration ranging from 16.8 to 17 days.[36,37]

Hospitalization length will be recorded from the day of admission (day 1) to the date of official discharge. This outcome is similarly grounded in evidence from recent meta-analyses reporting a mean hospitalization duration

exceeding 10 days in COVID-19 patients, which may vary according to age, comorbidities, and available resources.[38]

6.2. Secondary study outcomes

Body temperature will be measured orally using a calibrated digital thermometer, as oral thermometry is a widely accepted clinical method offering a practical balance between patient comfort and reliability. Assessments will be conducted on days 1 (baseline), 5, 7, 10, 14, 15, 16, and 17. If a patient's body temperature returns to normal and remains so for 48 h, then no further temperature measurements will be conducted.

The severity and duration of patients' coughs will be assessed through a cough Visual Analog Scale, which will be validated for both acute and subacute cough conditions.[39,40] In addition, a revised Leicester Cough Questionnaire (LCQ)[41] will be used for the assessment of cough-specific QoL, altering its duration to 2 days (Figure A1). The time point of the initial evaluation will be day 1 (baseline), and the revised LCQ will be reassessed for patients every second day for 20 days. If cough resolution occurs earlier, the assessments will be ceased. Its responsiveness will be determined by calculating the effect size of the change between baseline and cough resolution.

Other study outcomes include the assessment of the safety of the intervention and mortality rates. For these outcomes, a calculation and percentage comparison of adverse and serious adverse events, as well as mortality rates among trial arms, will be conducted. The time frame for these assessments will be set to 1 – 30 days.

7. Intervention, chemical characterization, and quality assurance

7.1. Intervention

The intervention will consist of a standardized 20% (w/v) hydroalcoholic extract of Greek poplar-type propolis, intended to be produced under Good Manufacturing Practice conditions. Hydroalcoholic extracts outperform other extraction types (e.g., aqueous, oil-based, supercritical) in terms of flavonoid and polyphenol yield, particularly when employing ethanol: water ratio of 70:30 with a solvent-to-propolis ratio of 5:1, which maximizes bioactive content and yields wax-free tinctures.[42-48]

Propolis will be sourced from the Imathia region of Greece, known for its high phenolic content and bioactive flavonoids with demonstrated *in silico* activity against SARS-CoV-2.[16] This selection is further supported by the findings of Kasiotis *et al.*,[16] who identified the Imathia region's propolis as having the highest total phenolic and flavonoid content among Greek samples, along with strong antioxidant activity comparable to quercetin.[16] Mediterranean propolis is also notable for its diterpene content, compounds with documented antibacterial and anti-inflammatory properties.[49,50]

Extraction will be conducted through ultrasound-assisted extraction (UAE) at 100% amplitude for 30 min at 58°C. Before extraction, crude propolis will be stored at −20°C in the dark to preserve its bioactive integrity.[51] The raw material will then be ground into powder, and UAE at 100% amplitude will be used as indicated above, in a hydroalcoholic mixture (ethanol: water) 70:30 v/v, in a solvent-propolis ratio (w/v) 1:5.[46] UAE combines mechanical and thermal effects that enhance solvent penetration, disrupt cellular structures, and significantly improve the yield of polyphenols compared to conventional maceration techniques.[52-55] Following filtration and re-extraction, the combined supernatants will be spray-dried, a method shown to preserve and even enhance the antioxidant activity and phenolic content of propolis compared to vacuum-drying alternatives.[56,57] This approach allows for alcohol-free, tasteless capsules that enhance palatability and enable indistinguishable placebos, supporting the integrity of the triple-blind design.

The resulting powder will be encapsulated into hard gelatin capsules delivering 800 mg/day over 10 days, based on dosing used in prior COVID-19 clinical trials without reported adverse events.[14] Placebo capsules will be identical in appearance and composition, excluding active propolis, as conducted by other researchers.[58]

7.2. Chemical characterization

Before formulation, raw propolis will undergo scanning electron microscopy for morphological characterization and screening for contaminants. Qualitative chemical analysis will confirm the presence of key phytochemical classes, including flavonoids, glycosides, phenols, and terpenoids. Balsam yield will be calculated gravimetrically using Popova's validated protocol.[59] Specifically, 2 mL of the extract will be evaporated in a vacuum oven to constant weight, and the balsam yield will be calculated using the following formula in Equation I:

$$\text{Yield (\%)} = (\text{Weight of dry ethanolic extract}/\text{Weight of crude propolis}) \times 100 \quad \text{(I)}$$

This procedure, first proposed by Popova *et al.*[59] and subsequently used by others, ensures consistent estimation of extraction efficiency across batches.

Quantification will focus on three principal bioactive groups: flavones/flavonols, flavanones/dihydroflavonols, and total phenolics, reflecting the taxonomic signature of poplar-type propolis.[60] These

will be analyzed through spectrophotometry and high-performance liquid chromatography, with complementary gas chromatography-mass spectrometry or liquid chromatography-mass spectrometry employed to confirm compound identity and enhance resolution, particularly for minor flavonoids like 3-methyl pinobanksin.[61-65]

All procedures will adhere to the WHO and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) guidelines on investigational product quality and reproducibility.[66-68]

7.3. Quality control and assurance

The investigational product will comply with Good Manufacturing Practice standards, following guidelines set by the WHO and the ICH.[66,68] To ensure product quality, consistency, and reproducibility, independent laboratory analyses will be performed on both the raw material and the final formulation.

As no authorized pharmaceutical or nutraceutical product is being used, chemical characterization results – including chromatography and mass spectrometry data – will be provided as supplementary documentation, in alignment with best practices for investigational compounds.[68] Moreover, the study will follow the CONSORT guidelines to ensure methodological transparency and data integrity throughout the trial.

8. Sample size and randomization strategy

A priori power calculations based on Schoenfeld's formula (Figure A2)[69] indicated that approximately 330 events are required to achieve 80% statistical power at a 5% significance level, assuming a hazard ratio of 0.7 and adjusting for multiple comparisons using Bonferroni correction.[70,71] Allowing for a 25% censoring rate of the primary endpoint (hospitalization duration ≤20 days),[72] the total sample size was determined to be 441 participants.

Participants will be equally randomized to three arms – intervention, placebo, and control – in a 1:1:1 ratio (147 per group). Stratified randomization will be employed to control for two key prognostic factors: age and vaccination status, both of which significantly influence COVID-19 progression and outcomes.[38,73-75] For instance, unvaccinated individuals over 65 are approximately 4.5 times more likely to die from COVID-19 than their vaccinated counterparts,[74] while vaccinated individuals have shown shorter viral clearance times.[75]

To ensure allocation balance, participants will first be stratified by age (18 – 49, 50 – 64, and 65 – 85 years) and vaccination status (Table 1), yielding six strata based on United States Centers for Disease Control and Prevention reported hospitalization distributions (29%, 29%, and 42% per age group, respectively, with ~80% unvaccinated).[76,77] Random assignment within each stratum will be conducted using computer-generated sequences (Table 2). This approach mitigates confounding and strengthens the internal validity of treatment comparisons across groups.

Table 1. Strata, stratification variables, and sample size

Strata	Age	Sample's age group (%)	Vaccination status	Stratum (%)
Stratum 1	18 – 49	29	Vaccinated	20% of 29%
Stratum 2	18 – 49	29	Unvaccinated	80% of 29%
Stratum 3	50 – 64	29	Vaccinated	20% of 29%
Stratum 4	50 – 64	29	Unvaccinated	80% of 29%
Stratum 5	65 – 85	42	Vaccinated	20% of 42%
Stratum 6	65 – 85	42	Unvaccinated	80% of 42%

9. Statistical analysis

Statistical analysis will be performed in accordance with the intention-to-treat principle. All randomized participants who receive at least one dose of the assigned intervention or placebo will be included in the final analysis. Descriptive statistics will be used to summarize baseline demographic and clinical characteristics, with continuous variables expressed as means and standard deviations or medians and interquartile ranges, depending on the distribution, and categorical variables as frequencies and percentages.

Comparisons between the intervention and control groups will be performed using appropriate inferential tests based on data type and distribution. For continuous outcomes such as time to viral clearance, length of hospitalization, and biomarker changes (e.g., C-reactive protein, interleukin-6), the independent samples *t*-test will be used for normally distributed data, while the Mann–Whitney U test will be applied for non-normally distributed variables. For categorical variables such as the need for oxygen therapy and the occurrence of adverse events, Chi-square or Fisher's exact tests will be employed.

Kaplan–Meier survival analysis will be used to evaluate time-to-event outcomes, including time to viral clearance, with log-rank tests used to compare curves between groups. Multivariable Cox proportional hazards models may be considered to adjust for potential confounders such as age, comorbidities, and baseline severity scores.

A *p*-value of less than 0.05 will be considered statistically significant. Statistical analyses will be conducted using the Statistical Package for the Social Sciences (version 26.0; IBM Corp., United States) or equivalent statistical software.

Table 2. Number of participants per stratum

Strata	Age	Vaccination status	Number of participants	Number of participants per stratum (Rounded)	Total number of participants (Sample size)
Stratum 1	18 – 49	Vaccinated	25.52	26	441
Stratum 2	18 – 49	Unvaccinated	102.08	102	
Stratum 3	50 – 64	Vaccinated	25.52	26	
Stratum 4	50 – 64	Unvaccinated	102.08	102	
Stratum 5	65 – 85	Vaccinated	36.96	37	
Stratum 6	65 – 85	Unvaccinated	147.84	148	

Interim analyses are not planned for this study, given the relatively small sample size.

10. Limitations

A key limitation in propolis research is its chemical heterogeneity, which complicates large-scale standardization and clinical reproducibility.[60] This trial addresses the issue by classifying propolis as poplar-type based on the relative ratios of flavones/flavonols, flavanones/dihydroflavonols, and total phenolics. Although not as precise as patented methods, this chemotaxonomic approach enables batch comparability and may serve as a foundational model for future studies.

Standardized extracts such as EPP-AF® (Apis Flora, Brazil) offer batch-to-batch consistency through a patented identification and formulation system.[78,79] Comparative trials could further explore the clinical differences between such industrial-standardized formulations and the source-based model employed here.

Another limitation concerns the interpretation of vaccination coverage data in the context of the study. Reported figures often reflect individuals with only one vaccine dose and lack details on booster uptake or timing post-vaccination. Although these factors could influence baseline immunity and study outcomes, their inclusion in stratification would have added analytical complexity beyond the scope of this trial.

11. Conclusion

This study protocol presents the design of a randomized, triple-blind, placebo-controlled clinical trial investigating the therapeutic potential of a standardized Greek poplar-type propolis extract as an adjunct to standard care in hospitalized patients with COVID-19. Given the urgent need for accessible and evidence-based supportive treatments, propolis – a natural product with demonstrated antiviral, immunomodulatory, and anti-inflammatory properties – warrants rigorous clinical investigation. If implemented, this trial could generate valuable data regarding the clinical efficacy and safety of propolis in the context of acute SARS-CoV-2 infection and contribute to the broader discourse on integrative approaches to infectious disease management.

Moreover, given the evolving burden of post-viral syndromes such as long COVID, the immunomodulatory effects of propolis may hold additional therapeutic relevance beyond acute infection – a hypothesis that future trials may further elucidate.[80]

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

Conceptualization: Giorgos Tzigkounakis
Writing – original draft: All authors
Writing – review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Availability of data

Not applicable.

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Appendices

This questionnaire is designed to assess the impact of cough on various aspects of your life. Read each question carefully and answer by CIRCLING the response that best applies to you. Please answer ALL questions, as honestly as you can.

1. In the last 2 days , have you had chest or stomach pains as a result of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

2. In the last 2 days , have you been bothered by sputum (phlegm) production when you cough?

1	2	3	4	5	6	7
Every time	Most times	Several times	Some times	Occasionally	Rarely	Never

3. In the last 2 days , have you been tired because of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

4. In the last 2 days , have you felt in control of your cough?

1	2	3	4	5	6	7
None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

5. How often during the last 2 days have you felt embarrassed by your coughing?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

6. In the last 2 days , my cough has made me feel anxious

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

7. In the last 2 days , my cough has interfered with my job, or other daily tasks

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

8. In the last 2 days , I felt that my cough interfered with the overall enjoyment of my life

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

9. In the last 2 days , exposure to paints or fumes has made me cough

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

10. In the last 2 days , has your cough disturbed your sleep?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

11. In the last 2 days , how many times a day have you had coughing bouts?

1	2	3	4	5	6	7
All of the time (continuously)	Most times during the day	Several times during the day	Some times during the day	Occasionally through the day	Rarely	None

12. In the last 2 days , my cough has made me feel frustrated

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

13. In the last 2 days , my cough has made me feel fed up

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

14. In the last 2 days , have you suffered from a hoarse voice as a result of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

15. In the last 2 days , have you had a lot of energy?

1	2	3	4	5	6	7
None of the time	Hardly any of the time	A little of the time	Some of the time	A good bit of the time	Most of the time	All of the time

16. In the last 2 days , have you worried that your cough may indicate serious illness?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

17. In the last 2 days , have you been concerned that other people think something is wrong with you, because of your cough?

1	2	3	4	5	6	7
All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

18. In the last 2 days , my cough has interrupted conversation or telephone calls

1	2	3	4	5	6	7
Every time	Most times	A good bit of the time	Some of the time	A little of the time	Hardly any of the time	None of the time

19. In the last 2 days , I feel that my cough has annoyed my partner, family or friends

1	2	3	4	5	6	7
Every time I cough	Most times when I cough	Several times when I cough	Some times when I cough	Occasionally when I cough	Rarely	Never

Thank you for completing this questionnaire.

Figure A1. Study revised Leicester Cough Questionnaire

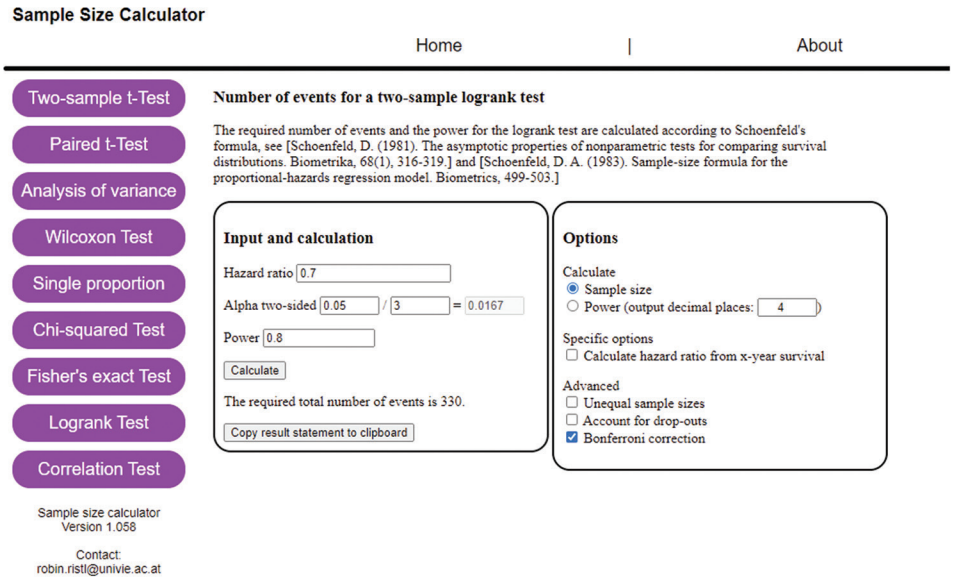

Figure A2. Power calculations based on Schoenfeld's formula
Notes: Schoenfeld's formula,[20] $n = (z_{1-\alpha} + Z_{\beta})^2/[\{\log(\lambda_1/\lambda_0)\}^2 P_1 P_0 d]$; Sample size calculation through *Sample size calculator,* version 1.058[21].
Source: https://homepage.univie.ac.at/robin.ristl/samplesize.php?test=logrank.

ACCSCIENCE PUBLISHING

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REVIEW ARTICLE

Functional gains through video-directed exercises in post-stroke patients: A systematic review

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Abstract

Background: With the advancement of technology and the increasing need for low-cost rehabilitation alternatives, video-demonstrated exercises have demonstrated potential as a viable solution for patients with limited access to in-person therapy. Post-stroke rehabilitation involves functional improvements in various areas, including structure and function, activity, and participation, as defined by the International Classification of Functioning (ICF), Disability, and Health. While in-person exercises have been widely studied, the use of audiovisual resources for promoting functional recovery is a more recent approach that requires further scientific investigation to confirm its effectiveness and benefits. The present study is a systematic review of the effects of video-demonstrated exercises in rehabilitating post-stroke individuals. **Aim:** This review aims to describe the functional improvements in structure and function, activity, and participation promoted by video-demonstrated exercises in post-stroke individuals. **Methods:** A search was conducted between October 2008 and December 2024 across three online databases: BVS, PubMed, and Web of Science. Only randomized clinical trials published in English, involving post-stroke patients and comparing video-based exercises with other physical therapy interventions, were included. Articles not available in full, duplicates, and those unrelated to the topic were excluded. **Results:** These studies revealed that video-based interventions provided similar functional improvements to traditional therapy, with no significant differences found between the groups. The limited number of studies and variations in intervention duration highlight the need for further research in this area. **Conclusion:** Guided rehabilitation programs produced comparable results to face-to-face therapy with functional improvements according ICF, Disability, and Health. **Relevance for patients:** Video-guided exercise rehabilitation programs have demonstrated comparable outcomes to face-to-face therapy after 3 months of therapy, with improvements in function and quality of life after stroke.

Keywords: Stroke; Audio-visual media; Instructional films and videos; Rehabilitation; Stroke

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1. Introduction

Stroke is considered a global health problem and a disabling condition.[1] According to the 2013 National Health Survey, there are more than two million stroke survivors in Brazil, approximately 568,000 of whom live with severe disability. This comorbidity is more prevalent in the elderly population, those living in urban areas, and those with lower educational levels, and factors such as obesity and physical inactivity increase the risk of its occurrence, raising concerns about the increase in the number of cases.[2,3] In addition, lifestyle changes have contributed to the rising incidence among the younger population, especially due to the association with hypertension.[4] As a chronic condition that results in functional deficits, individuals affected by stroke require ongoing care, even after hospital discharge.[5]

Rehabilitation treatment, typically provided in specialized units, aims to reduce secondary complications, promote recovery of bodily functions, increase independence in daily living activities, and contribute to social reintegration.[5,6] Nonetheless, various challenges, such as the high cost of rehabilitation, insufficient awareness of its benefits, and limited access to transportation, create substantial obstacles to effective patient care.[7] To address these issues, several care models have been developed to better align with the needs and expectations of patients, caregivers, and healthcare providers. In the case of stroke survivors, many express a preference for exercises demonstrated to them, as the visual presentation helps in the correct execution of movements, providing greater safety for both the patient and the caregiver.[8]

It is important to note that impaired balance can harm the autonomy and quality of life of both individuals who have suffered a stroke and their caregivers. Proprioceptive training has proven to be an effective strategy for recovering motor skills and preventing falls, but it requires hospitalization or daily in-person sessions, which can limit access for many patients.[9] Thus, video-assisted rehabilitation has proven to be a promising alternative, as it allows stroke survivors to follow targeted rehabilitation strategies in the comfort of their homes, contributing to the continuity of treatment, in addition to providing better conditions for access and adherence to the desired therapy.

Exercise demonstrations through video can offer even greater precision, allowing for detailed monitoring of movements, with the possibility to revisit the video as many times as needed for effective learning.[10] Studying the functional outcomes of video-based therapy can provide a deeper understanding of home rehabilitation and its effects on the patient, caregiver, and even healthcare professionals. The International Classification of Functioning (ICF), Disability, and Health suggests that individuals should be analyzed in a context that considers the interaction between their health condition, body structures and functions, activity performance, social participation, and contextual factors that may facilitate or hinder their functionality.[11,12] The main objective of this review is to describe functional improvements in the domains of structure and function, activity, and participation promoted through video-demonstrated exercises. As a secondary objective, the assessment instruments used in the studies will be listed.

2. Methods

This study is characterized as a systematic review, with the search conducted between October 2008 and December 2024 across three online databases: BVS (Virtual Health Library), PubMed (National Library of Medicine and National Institutes of Health), and Web of Science. To facilitate the search for evidence, the following question was formulated (PICO strategy): "What functional improvements can video-demonstrated exercises promote in post-stroke individuals?"; where the population consisted of individuals post-stroke, the intervention involved exercise programs demonstrated through audiovisual means, compared to other physical therapy interventions, and the outcomes were measured in the domains of structure and function, activity, and participation according to the ICF.

Articles published in English between 2008 and 2024 were included; randomized clinical trials (RCTs) with samples consisting of post-stroke patients allocated into two groups, with the experimental group receiving only video-based exercises as the intervention, were selected. Articles not available in full, duplicate articles across databases, those not addressing the core topic, as well as monographs and theses, were excluded.

The search was conducted by two blinded researchers using the following descriptors and their synonyms: "Neurological Rehabilitation" and "Stroke Rehabilitation," combined with "Instructional Films and Videos," "Video-Audio Media," and "Telerehabilitation," excluding those related to virtual reality. All terms are registered in the National Library of Medicine's controlled vocabulary thesaurus (MeSH) and the Health Sciences Descriptors (DeCS). The Boolean operator "OR" was used between the descriptors and their synonyms, while the Boolean operator "AND" was used between different descriptors. Articles were initially screened by reading titles and abstracts, followed by an examination of full texts. After each of these steps, a third researcher was consulted to resolve discrepancies between results and decide whether to retain or exclude articles. This rigorous analysis allowed the identification of relevant studies, despite challenges

such as the small number of studies directly addressing the research question.

3. Results

A total of 1,910 articles were identified in the initial search of the databases. After thorough screening, only four studies met the inclusion criteria for this review. The selection process is illustrated in Figure 1. The exclusion of 1,903 studies was based on the following reasons: They did not use video-based exercises in the therapy applied to the intervention groups; they were pilot studies that had not yet been implemented; they were not RCTs; or their populations did not consist of post-stroke patients.

The characteristics of authors, outcomes, interventions, and results of the selected works are detailed in Table 1.

Table 2 presents the assessment of the risk of bias of the studies included in the review using the physiotherapy evidence database scale, which is a scale with acceptable reliability and considered suitable for assessing the methodological quality of RCTs.

Emmerson *et al.*[10] conducted an RCT comparing home exercise programs for post-stroke patients using smart technology (videos and automated reminders) with traditional paper-based programs. Participants were divided into two groups: One group received written exercise instructions, while the other used a tablet for video-based exercises with personalized feedback from a therapist. After 4 weeks, no significant differences were found between the groups regarding adherence, satisfaction, or upper limb function improvement as measured by the Wolf Motor Function Test (WMFT).

Similarly, Redzuan *et al.*[13] assessed the effectiveness of a video-guided home exercise program for post-stroke individuals. Ninety participants were assigned to a control group (weekly in-person therapy) or an intervention group (video-guided exercises). After 3 months, both groups demonstrated comparable improvements in the Barthel Index, reduced complications, and less caregiver stress, with no significant differences between the two.

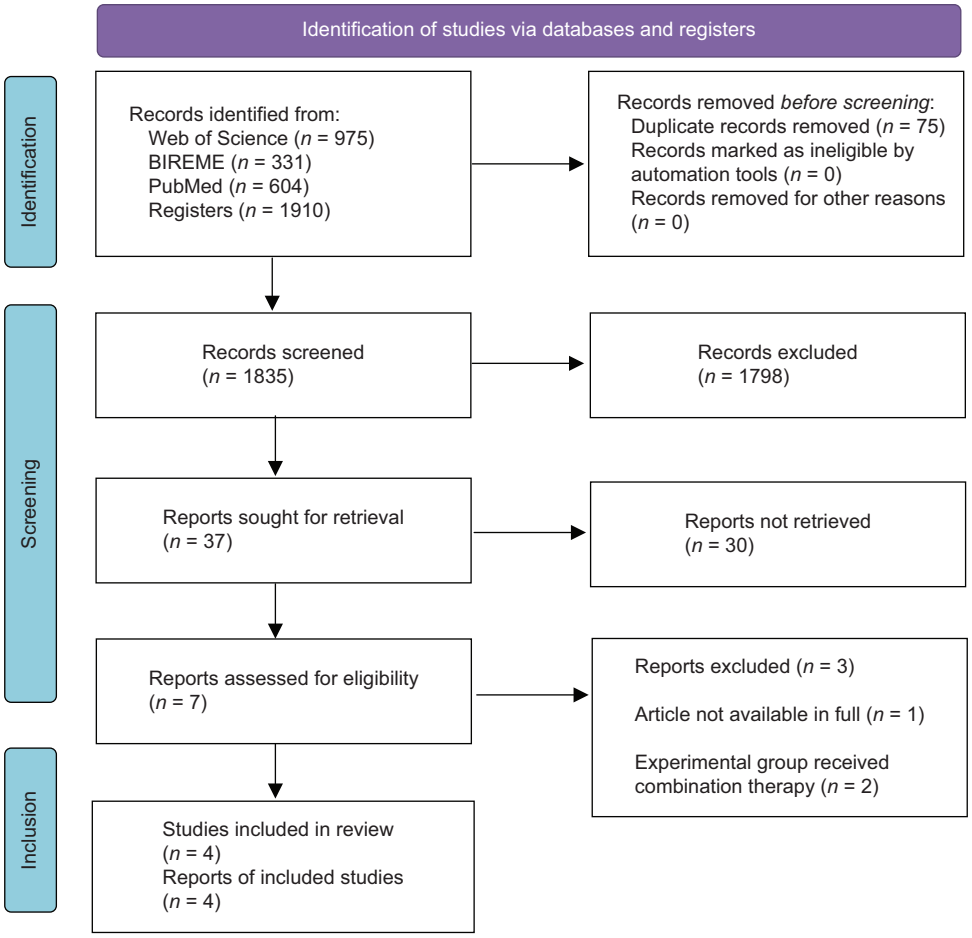

Figure 1. Preferred reporting items for systematic reviews and meta-analyses flowchart

Table 1. Articles included in the review

Authors (year)	Sample	Outcome	Assessment instrument	Intervention	Results
Emmerson *et al.* (2017)[10]	62 subacute (120 days) post-stroke individuals with a functional deficit in upper limbs; mean age (SD): 66 (16)	To determine whether patients have better adherence to a home exercise program when they receive technological support compared to a paper prescription	Self-report diary, Wolf Motor Function Test, and satisfaction questionnaire developed by the authors	Duration: 4 weeks Control: Instructions for the exercises in written format Intervention: Home exercises delivered via tablet, instructed, recorded, and commented on by the therapist; images were of the patients themselves	There was no significant difference between the groups in adherence and satisfaction levels, Wolf Motor Function Test indices, and the frequency of performing the exercises
Redzuan *et al.* (2012)[13]	90 acute post-stroke individuals Intervention group (mean age [SD]): 63.7 (12) Control group (mean age [SD]): 59.4 (11)	To evaluate the effectiveness of an intervention that uses video in a home exercise program for people after a stroke	Modified Barthel Index (MBI), caregiver strain index	Duration: 3 months Control: weekly professionally guided therapy Intervention: Video-guided therapeutic exercises and instructions	There was no significant difference between the groups; similar improvement among participants in the Barthel Index; reduction in the rate of complications and stress level of caregivers
Asano *et al.* (2019)[14]	124 individuals with acute stroke Intervention group (mean age): 63.8 Control group (mean age): 64.4	To evaluate the impact of an innovative telerehabilitation intervention on self-reported functional outcomes compared with usual care during the first 3 months after stroke	Late-life function and disability instrument (LLFDI); the timed 5-m walk test; 2-min walking distance; MBI; the Activities-Specific Balance Confidence (ABC) scale; the EuroQoL (EQ-5D)	Duration: 3 months Control: Weekly 1-h rehabilitation sessions (once or twice a week), depending on the needs of each patient, considered as usual care for this study Intervention: Access to a telerehabilitation system and a standardized rehabilitation program for 3 months, and a therapist determined the level of difficulty and minimum range of motion desired for each exercise, according to the individual needs of each patient	Both groups displayed similar improvements in all outcomes; no significant differences were seen in the disability instrument (LLFDI) in either group
Chen *et al.* (2020)[15]	52 individuals with acute stroke Intervention group (mean age [SD]): 64.19 (9.42) Control group (mean age [SD]): 59.42 (10.0)	To determine the effects of a 12-week home-based motor-training telerehabilitation procedure in subcortical stroke patients with motor dysfunction by the combined use of motor function assessments and multimodal magnetic resonance imaging analysis methods	Fugl-Meyer assessment (FMA) for upper and lower limbs; MBI	Duration: 12 weeks Control: Completed in-person rehabilitation training in the outpatient rehabilitation department Intervention: Participated in home-based rehabilitation training with the telemedicine rehabilitation system (TRS) under the guidance of therapists; therapists supervised patients to conduct therapy via live videoconferencing	From the results of the superiority test, a significant difference was found for change in FMA score in a one-tailed test (p=0.011), with 97.5% CI of 0.076 – 7.456, and no difference was observed for change in MBI score (97.5% CI: 0.0856 – 11.098; p=0.097)

Abbreviations: SD: Standard deviation; CI: Confidence interval.

Asano *et al.*[14] investigated the effects of an innovative telerehabilitation intervention on self-reported functional outcomes during the first 3-month post-stroke. In the study, 124 individuals were assigned to either a telerehabilitation group (standardized rehabilitation system with therapeutic supervision) or a control group (weekly in-person sessions). Both groups demonstrated similar improvements, with no significant differences in functional outcomes.

Table 2. Risk of bias using the physiotherapy evidence database (PEDro) scale

PEDro scale questions	Studies included in the review			
	Emmerson *et al.* (2017)[10]	Redzuan *et al.* (2012)[13]	Asano *et al.* (2019)[14]	Chen *et al.* (2020)[15]
Subjects were randomly allocated to groups (in a crossover study, subjects were randomly allocated an order in which treatments were received)	Yes (+1)	Yes (+1)	Yes (+1)	Yes (+1)
Allocation was concealed	Yes (+1)	No (0)	Yes (+1)	Yes (+1)
The groups were similar at baseline regarding the most important prognostic indicators	Yes (+1)	No (0)	Yes (+1)	Yes (+1)
There was blinding of all subjects	No (0)	No (0)	No (0)	No (0)
There was a blinding of all therapists who administered the therapy	No (0)	No (0)	No (0)	No (0)
There was blinding of all assessors who measured at least one key outcome	Yes (+1)	No (0)	No (0)	Yes (+1)
Measures of at least one key outcome were obtained from more than 85% of the subjects initially allocated to groups	Yes (+1)	No (0)	Yes (+1)	Yes (+1)
All subjects for whom outcome measures were available received the treatment or control condition as allocated or, where this was not the case, data for at least one key outcome was analyzed by "intention to treat"	No (0)	No (0)	Yes (+1)	Yes (+1)
The results of between-group statistical comparisons are reported for at least one key outcome	Yes (+1)	Yes (+1)	Yes (+1)	Yes (+1)
The study provides both point measures and measures of variability for at least one key outcome	Yes (+1)	Yes (+1)	Yes (+1)	Yes (+1)
Total score	7/10	3/10	7/10	8/10

Chen *et al.*[15] explored the impact of a 12-week home-based motor telerehabilitation program for subcortical stroke patients. In the study, 52 individuals were divided into a telerehabilitation group (supervised videoconference training) and a control group (in-person outpatient rehabilitation). The telerehabilitation group displayed significant gains in Fugl-Meyer Assessment (FMA) scores (p=0.011), indicating improved motor function, but there were no significant differences in modified Barthel index (MBI) scores.

These findings suggest that rehabilitation programs incorporating video-guided exercises can yield results comparable to face-to-face therapy, offering a safe and cost-effective alternative for stroke survivors. Functional improvements were observed across all ICF domains, though most studies focused on activity as the primary outcome. Future research exploring changes in social participation and functional outcomes may provide a deeper understanding of clinical progression. Video-based approaches could potentially reduce social disparities and enhance access to comprehensive stroke care.

The limited number of RCTs in this area highlights the need for further studies with clear, high-quality methodological designs to ensure their findings can be effectively translated into clinical practice. Variations in intervention duration and intensity may have influenced the results, as shorter interventions[10] have less impact compared to longer programs.[13-15]

Personalized feedback plays a crucial role in enhancing the efficacy of home-based programs. Both Emmerson *et al.*[10] and Chen *et al.*[15] incorporated therapist feedback, which has the potential to increase engagement and adherence. However, despite this personalization, the results did not consistently exhibit significant advantages over traditional methods. This suggests that while technology offers convenience, the quality of interaction between patients and therapists needs further refinement to optimize outcomes in home-based stroke rehabilitation.

Although technological advancements such as video-guided exercises and automated reminders have been integrated into home rehabilitation programs, the reviewed studies suggest that these innovations alone may not significantly improve outcomes. For instance, Emmerson *et al.*[10] and Redzuan *et al.*[13] found no significant differences in rehabilitation success compared to traditional methods. This suggests that factors beyond the mode of delivery, such as intensity, engagement, and the individualized nature of the interventions, play a crucial role in determining the effectiveness of home-based stroke rehabilitation programs. Future studies should explore how to optimize the interaction between technology, patient engagement, and therapist involvement in remote settings.

The lack of significant differences between telerehabilitation and in-person therapies raises the potential of both approaches to deliver effective

rehabilitation. However, the absence of marked improvements in functional outcomes, such as the BI and FMA, raises questions about the sensitivity of these assessments to detect subtle but clinically meaningful changes. Further research could benefit from using more specific or comprehensive outcome measures to capture a broader range of functional and quality-of-life improvements. This would allow for a deeper understanding of how different rehabilitation models impact long-term recovery and whether combining elements of both telehealth and traditional therapies could optimize post-stroke rehabilitation.[13,15]

A common limitation across these studies is the reliance on relatively short-term follow-up periods (1 – 3 months). While these studies provide valuable insights into immediate and short-term rehabilitation outcomes, the long-term effects of home-based rehabilitation programs remain underexplored. Longitudinal studies tracking patients' progress over extended periods are needed to assess the sustainability of functional improvements, long-term independence, and the overall impact of these interventions on stroke survivors' quality of life. These insights are essential to refining home rehabilitation programs to ensure that they improve both short-term recovery and long-term health benefits.

4. Discussion

This review provides a state-of-the-art review of the evidence on improvements in the functionality of individuals who have suffered a stroke after performing exercises demonstrated through video. Despite the small number of articles found, there was a consensus that the use of video-based exercises is capable of achieving results similar to conventional therapy, performed in person by health professionals. Therefore, the choice to use this type of assistive technology is justified because it allows for a greater scope of rehabilitation programs, considering that many health services have a demand greater than the number of professionals available, in addition to offering patients and their caregivers greater convenience and ease.[10,13]

Rehabilitation in the home environment has been considered useful for improving the functional capacity of post-stroke individuals in a less costly manner for the patient and with the benefit of alleviating stress for caregivers.[1] However, its indication should be judicious, as it depends on personal conditions, such as educational level, age, and severity of the stroke, and is not recommended when the caregiver is absent or has little commitment and if there is no communication with the health team.[1,13] In addition, the perspective of the health provider should also be considered, as there may be additional costs for equipment, travel, and training of professionals.[13]

As one of the therapeutic strategies that can be delivered at home, audiovisual resources are capable of providing greater precision in movements, reinforcing correct exercise techniques, improving caregivers' care skills, and acting as reminders to maintain regular therapy.[10,13] This implies increased caregiver confidence during activities and increased therapy safety.

Such benefits were verified by Emmerson *et al.*,[10] who identified the use of videos in home rehabilitation programs as viable and safe, resulting in improvements in upper limb function. The use of this technology optimizes professional performance by allowing greater ease in adapting the exercise protocol throughout therapy, with the development of an intensity progression being recommended to avoid system accommodation.[10,13]

Individuals and caregivers experience worry and anxiety after a cerebrovascular event.[16] Evidence indicates that interventions that prepare caregivers for the new routine can alleviate feelings of burden and psychological stress.[16,17] It is also recommended that caregivers' knowledge and skills should be assessed before therapy to include their needs in the therapeutic planning.[18] Redzuan *et al.*[13] pointed out the reduction of burden on caregivers as one of the factors responsible for the better performance of activities of daily living in individuals in the acute phase after stroke who received exercise instructions via video.

The results presented also demonstrate the similarity of telerehabilitation with face-to-face therapy regarding the time spent performing exercises, as discussed by Asano *et al.*[14] In addition, the implementation of this practice in the rehabilitation of stroke patients may be a viable alternative to reduce transportation costs with hours of face-to-face supervision by health professionals. Another interesting finding was seen in Chen *et al.*,[15] who, in addition to research focused on motor function and degree of independence, also evaluated imaging biomarkers of neurological function to analyze and compare neural plasticity with recovery of function, obtaining a positive correlation of findings, thereby suggesting further studies in this perspective.

In the study by Redzuan *et al.*,[13] functional improvements were assessed by a difference of 30 or more points in the MBI after 3 months of intervention. The elements of the MBI present a moderate-to-strong correlation with the ICF, except for the items "anal continence" and "bathing," suggesting that it is an appropriate instrument to assist in the application of the ICF.[19] However, further studies with a larger sample size are warranted to better substantiate this

correlation.[18] Like the MBI, the WMFT was used to assess the activity domain.[10] However, the scale determines the ability to move only the upper limbs, analyzing the quality, speed, and strength of execution during the activities tested. Thus, it also includes the structure and function domains.[20]

There is a tendency to use assessment instruments that address the activity domain, as they facilitate the establishment of relationships with functionality and clinical reality.[10,13,21] For example, a deficit in a structure is often only noticed when it interferes with the execution of a task or social interaction. However, it is recommended that instruments should be chosen carefully. For the assessment of people with mild and moderate disabilities, investigation of instrumental activities and more complex tasks is recommended, avoiding the ceiling effect that is common when examining basic activities. While instruments that measure structure and function are the most capable of providing a rich comparison between pre- and post-intervention results, the measurement of participation still needs to be further researched and has been used as a secondary outcome.[21]

Attention and learning deficits are also analyzed with recurrence as a secondary outcome in interventions with exercise programs, but they have a great influence on the rehabilitation process. Attention is essential for the retention, organization, and execution of information and is indispensable for the learning process.[22] Video exercises contribute to this process, as they provoke the need for observation to perform the task, expanding the individual's repertoire of activities through mistakes and successes, in addition to generating new motor patterns.[21,22] This observe-execute sequence is repeated in other therapeutic approaches, such as in action observation therapy, and is justified by the activation of mirror neurons after the execution or observation of a task.[23-28]

It is important to understand that stroke causes several consequences for the individual and affects their quality of life and functionality.[11,24,29] Considering the relationship between the elements that make up the ICF when investigating interventions demonstrated by video can enable a clear organization of information and facilitate application in clinical practice, in addition to contributing to the development of strategies aimed at comprehensive rehabilitation. Home-based approaches through videos allow patients to carry out rehabilitation comfortably, overcoming some barriers that could interfere with their adherence to treatment.[7,10,13]

Notably, one of the articles included in this review presents a confusing methodology that makes the intervention difficult to understand and hinders its replication in future studies. However, it indicates the viability of using video exercises as a way of learning and monitoring stroke patients who have functional deficits in the upper limb.[10,30-33]

One of the key findings of this review is the potential for video-based interventions to reduce the barriers to accessing rehabilitation, particularly for individuals living in rural or underserved areas. The use of video exercises facilitates adherence to treatment and provides flexibility, allowing patients to follow their rehabilitation schedules without the need for frequent in-person visits. This approach may be especially beneficial in settings where healthcare professionals are scarce, ensuring that patients still have access to structured rehabilitation programs. The ability to access rehabilitation exercises at home also mitigates the logistical challenges of travel, potentially increasing adherence to prescribed exercise regimens, thereby leading to better outcomes for stroke patients. This aspect of home-based telerehabilitation is an advantage that may improve stroke rehabilitation accessibility, especially when coupled with ongoing support from healthcare providers.

In addition, the positive correlation between the use of video-guided exercises and improved functional outcomes, as observed in some studies, highlights the potential of these interventions to support neurological recovery. The findings from Chen *et al.*,[15] which indicated a link between neural plasticity and recovery of function, provide a compelling argument for the use of video-based therapies in rehabilitation programs. These results suggest that home-based rehabilitation, when delivered through video-guided exercises, might not only support motor function but also contribute to neuroplastic changes that are crucial for recovery after stroke. Further studies investigating the neurophysiological underpinnings of video-based rehabilitation would be valuable in understanding the full extent of these interventions' benefits on neural recovery.

The ability of video interventions to enhance caregiver involvement is another important aspect highlighted by the studies reviewed. As caregivers play a critical role in the rehabilitation process, especially in home-based settings, the educational component embedded in video interventions can empower caregivers with the necessary skills and confidence to assist in the rehabilitation process. This is particularly important in post-stroke recovery, where the patient's functional abilities can fluctuate, and caregivers may face challenges in providing appropriate care. As reported by Redzuan *et al.*,[13] the reduction of caregiver burden is closely linked to improved patient outcomes, underscoring the importance of incorporating caregiver training into rehabilitation programs. Video-based interventions provide an effective platform for

such education, offering real-time visual demonstrations of exercises and techniques that caregivers can then implement, leading to better overall management of stroke recovery at home.

Despite the promising results, some limitations need to be addressed in future research. One of the major concerns is the variability in the intensity and duration of the interventions across studies, which could influence the outcomes. While some studies employed short-term interventions, others used longer rehabilitation periods, making direct comparisons difficult. The optimal duration and intensity of video-based rehabilitation programs remain unclear, and future studies should focus on determining the most effective parameters for these interventions. In addition, the role of patient engagement, including factors such as motivation and self-monitoring, warrants further exploration. Personalized feedback, as noted in some studies, may enhance engagement, but its impact on long-term adherence and outcomes requires more comprehensive analysis.

Another challenge highlighted by this review is the lack of long-term follow-up in most studies. While short-term improvements in motor function and daily living activities are promising, it remains uncertain whether these benefits are sustained over time. Long-term follow-up is essential to determine the durability of the rehabilitation effects and to assess whether video-based interventions can contribute to sustained improvements in quality of life and independence for stroke survivors. Moreover, future studies should include a broader range of outcome measures that capture not only physical function but also psychological and social outcomes, which are equally important for overall recovery. Understanding how video-based rehabilitation influences these domains will provide a more comprehensive view of its potential benefits.

Another critical aspect that warrants further attention is the accessibility and technological requirements for video-based rehabilitation interventions. While video-guided exercises can enhance the flexibility and reach of rehabilitation programs, they may not be universally accessible to all stroke survivors. Issues such as internet connectivity, access to suitable devices (e.g., smartphones, tablets, or computers), and technological literacy can limit the effectiveness of such interventions, particularly in low-resource settings or for older adults with limited digital skills. Future studies should explore how to overcome these barriers, potentially through partnerships with community health organizations or the development of low-cost solutions. Ensuring that video-based rehabilitation is accessible to all individuals, regardless of socioeconomic status or technological proficiency, will be key to maximizing its impact.

Lastly, the role of interdisciplinary collaboration in implementing video-based rehabilitation should not be overlooked. Stroke recovery is complex and often requires the expertise of various healthcare professionals, including physiatrists, neurologists, physical therapists, and occupational therapists. Video-based interventions could be most effective when they are part of a comprehensive rehabilitation plan that incorporates input from multiple disciplines. Collaborative care ensures that stroke survivors receive holistic treatment, addressing not only physical function but also cognitive, emotional, and social aspects of recovery. Future research should focus on the development of integrated care models that combine video interventions with in-person therapy, leveraging the strengths of both approaches to optimize recovery and improve patient outcomes.

Incorporating video-guided exercises into physical therapy programs for stroke survivors has the potential to significantly enhance rehabilitation outcomes, particularly when integrated with traditional in-person physical therapy sessions. Video-based exercises can complement these in-person sessions by providing stroke patients with continuous access to therapeutic content outside the clinic, helping them maintain consistency in their rehabilitation. The use of videos as a supplement to physical therapy could also allow therapists to track patients' performance remotely, offering feedback and adjustments as necessary, which may help patients stay motivated and adhere to their rehabilitation plans. The application of video-guided exercises in stroke rehabilitation is not limited to the home setting alone. Healthcare facilities, including outpatient clinics, rehabilitation centers, and even community health programs, can also benefit from integrating this technology into their rehabilitation practices.

Another significant advantage of video-guided exercise programs is their potential for application in remote or underserved areas, where access to skilled healthcare professionals may be limited. Patients in rural or remote locations can receive high-quality, evidence-based rehabilitation from the comfort of their own homes, without the need for frequent travel. Furthermore, healthcare providers in these areas can use video programs to remotely monitor patients' progress, providing virtual feedback and adjustments to the exercise routines, thus ensuring continuous support and optimizing outcomes.

The main limitation of this study was the small number of articles included that addressed the research question. This can be explained by the restriction of including only RCTs

and video interventions that contained exercise programs. Since the aim of this article was to facilitate the visualization and application of the results in professional practice, only randomized trials were used. Another limitation concerns the understanding of the methodological design of one of the included articles, which made it difficult to correlate it with the other included studies.

Furthermore, the studies included in this review performed therapy for post-stroke individuals in the acute or subacute phase, and it is not possible to generalize these findings to individuals in the chronic phase. These data suggest the importance of early intervention in this population, with a greater possibility of functional gains. Further studies employing telerehabilitation for individuals in the chronic phase of stroke are warranted for a more detailed investigation of the potential effects on functional capacity and quality of life.

5. Conclusion

Post-stroke rehabilitation plays a crucial role in the functional recovery of patients and remains a constantly evolving field. The integration of new therapeutic approaches, such as telerehabilitation programs and home therapy, has displayed significant benefits, providing greater functionality and independence for patients. These alternatives demonstrate results comparable to traditional care, promoting treatment adherence in a more accessible and flexible manner. Furthermore, they help reduce barriers to rehabilitation access, making treatment more feasible for individuals in remote areas or with mobility difficulties.

In addition, the active participation of family members and caregivers in the recovery process is essential for the success of treatment. The emotional and physical support provided by family members, along with direct involvement in the therapeutic process, can accelerate recovery and reduce the stress associated with caregiving. Developing strategies that engage the support network in a structured way is crucial to optimizing rehabilitation outcomes and creating a continuous and motivating environment for the patient. Moreover, it would be interesting to develop research that analyzes changes in the function and social participation of these individuals, in addition to RCTs on post-stroke rehabilitation.

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Conflict of interest

The authors declare that they have no competing interests.

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ORIGINAL ARTICLE

Non-invasive early detection of cervical carcinogenesis through the olfactory response of *Caenorhabditis elegans*

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Abstract

Background: Human papillomavirus (HPV) is the primary cause of cervical cancer, often through the development of cervical intraepithelial neoplasia (CIN). Persistent infection with high-risk HPV types can lead to severe dysplasia and invasive cancer. Early detection of progressive cervical carcinogenesis is crucial for improving outcomes. By targeting CIN, a pre-cancerous stage, therapeutic interventions are most effective and least invasive, offering the potential to reduce the incidence of invasive cervical cancer drastically. Early cervical carcinogenesis detection is hindered by inadequate screening coverage due to barriers, inaccurate screening methods, and patient compliance issues. Optimizing the timing, frequency, and technology availability of screening in resource-limited settings also poses significant challenges. **Aim:** This study evaluates the efficacy of Nematode-NOSE (N-NOSE), a novel *Caenorhabditis elegans*-based olfactory cancer screening tool, in detecting early cervical carcinogenesis. **Methods:** Urine specimens from 74 patients with cervical cancer and 245 patients with CIN were analyzed, with institutional review board approval from the National Hospital Organization Shikoku Cancer Center Hospital, Ehime, Japan. **Results:** We discovered that urine samples from CIN patients were successfully predicted to be positive using the N-NOSE test, with a sensitivity of 73% (180/245 patients) and 100% (9/9 patients) for CIN and pre-cancerous CIN3, respectively. **Conclusion:** N-NOSE demonstrates high sensitivity in detecting both early-stage CIN and invasive cervical cancer, suggesting its potential as a non-invasive, urine-based screening tool for early detection. This advancement also holds the promise of significantly improving preventive healthcare by enabling timely identification and intervention, leading to more efficient treatment modalities that effectively halt the progression of cervical carcinogenesis. **Relevance for patients:** The N-NOSE test offers a non-invasive, urine-based method for early detection of cervical cancer and pre-cancerous changes, enabling timely intervention and potentially enhancing patient outcomes.

Keywords: *Caenorhabditis elegans*; Cervical carcinogenesis; N-NOSE; Olfactory response

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1. Introduction

According to the World Health Organization (WHO), around 660,000 new cases of cervical cancer were diagnosed globally in 2022; with 94% of the 350,000 related deaths occurring in low- and middle-income countries.[1] This statistic highlights the critical need for ongoing efforts in prevention, screening, and treatment strategies to improve women's health.

The etiology of cervical cancer is closely linked to human papillomavirus (HPV) infection, with high-risk strains HPV-16 and HPV-18 responsible for most high-grade cervical pre-cancers.[2,3] These strains play a pivotal role in the disease's pathogenesis by integrating their DNA into host cells and disrupting normal cell cycle regulation. As illustrated in Figure 1, cervical carcinogenesis typically begins with the development of cervical intraepithelial neoplasia (CIN), progressing from mild dysplasia (e.g., CIN1) to more severe stages, such as CIN3, which is considered a pre-cursor to invasive cervical cancer. This study focuses particularly on the detection of CIN and CIN3, as these stages represent critical windows for intervention before the disease progresses to invasive cancer.[4,5]

While most cases of mild dysplasia, such as CIN1 or low-grade squamous intraepithelial lesions, may resolve due to the host's immune response, CIN2 and CIN3 (known as high-grade squamous intraepithelial lesions) infected with high-risk HPV types are considered true pre-cursor states for cervical cancer.[6-8]

Although HPV vaccination and traditional screening methods, such as cytology and histology, have contributed to prevention efforts, a significant disparity in the global burden of cervical cancer remains.[9] Early detection of CIN, representing the transformational phase of cervical cells, is pivotal in preventing CIN progression to invasive cervical malignancy, thereby improving patient prognosis and survival rates.[10,11] As a pre-cancerous condition, CIN offers a critical window for intervention that can halt the disease advancement.[12]

The early detection of cervical carcinogenesis is hindered by several factors, including inadequate screening coverage due to geographical, economic, and cultural barriers, as well as the accuracy limitations of screening methods, which may result in false negatives or positives. Additional challenges include ensuring patient compliance with follow-up care, determining optimal timing and frequency of screenings, and the availability of advanced screening technologies in resource-limited settings.

Recent advancements in biological diagnostics have focused on cancer detection, including the use of animal olfactory systems. While trained detection dogs have shown promise, their practicality is limited by the extensive training requirements.[13-16] An alternative is *Caenorhabditis elegans*, a well-studied nematode with a fully sequenced genome. Its robust olfactory system, comprising about 1,200 olfactory receptor-like genes,[17,18] enables sophisticated chemotactic behavior. Hirotsu *et al.*,[19] discovered that wild-type *C. elegans* are more attracted to cancer cell secretions, tissues, and urine from colorectal, gastric, and breast cancer patients while avoiding healthy urine samples. This response is possibly due to olfactory sensory neurons detecting cancer-associated volatile organic compounds (VOCs).[20,21]

Building on this concept, our research team developed an innovative cancer screening tool called "Nematode-NOSE" (N-NOSE). This method has successfully detected over 20 types of malignancies, including the most common types of cancer worldwide, such as lung, stomach, colorectal, breast, cervical, and prostate cancers, as well as various digestive cancers – including esophagus, bile duct, gallbladder, and pancreas cancers.[19,22-24] The present study evaluates the efficacy of N-NOSE in detecting progressive cervical carcinogenesis. Using urine samples from 74 female patients with cervical cancer and 245 female patients with CIN, we investigate the potential of N-NOSE to detect CIN as a pre-cancer stage with high sensitivity, as well as its ability to identify cervical cancer. This research contributes to the growing evidence supporting nematode-based cancer screening methods and explores their potential application in cervical cancer detection and prevention. Our research also aims to establish N-NOSE as a reliable, non-invasive diagnostic tool with heightened sensitivity, which could revolutionize screening and early detection in cervical health.

2. Materials and methods

2.1. Study design and participants

This study was approved by the Ethics Committee of the National Hospital Organization Shikoku Cancer Center Hospital, Ehime, Japan. The study design involved the prospective collection of urine samples from a cohort of female patients diagnosed with cervical cancer (n = 74, mean age: 51.9 ± 13.4 years) or CIN (n = 245, mean age: 39.5 ± 11.8 years) at the hospital between May 2017 and March 2021. Patients in the cervical cancer cohort were histopathologically confirmed to have invasive disease. In contrast, those in the CIN cohort were found to have pre-invasive lesions, identified through standard cervical cytology and subsequent detailed examinations, and classified according to the Bethesda System 2001 as CIN1, CIN2, or CIN3.[25] For the purpose of statistical analysis,

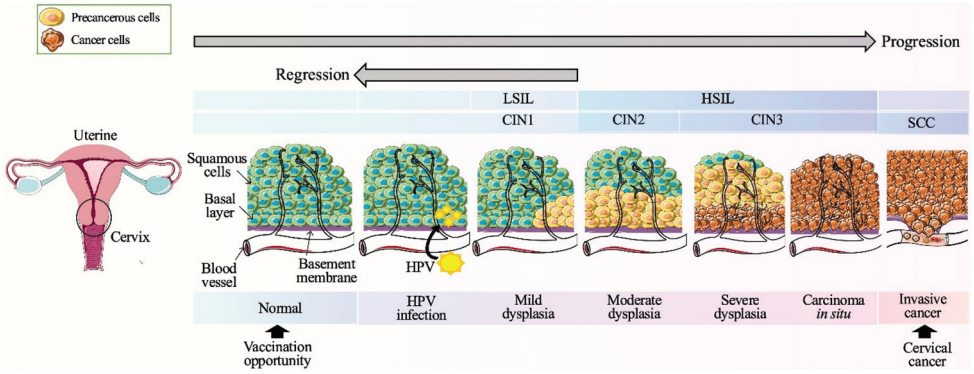

Figure 1. Cervical cancer progression. The schematic illustrates the sequential stages of cervical oncogenesis, from HPV infection to invasive SCC. Persistent HPV infection can trigger the progression of CIN stages, characterized by increasing dysplasia. Untreated high-grade CIN (such as CIN2 and CIN3) can progress to SCC, which invades the basement membrane and deeper tissues. Image created by the authors with Microsoft PowerPoint.
Abbreviations: CIN: Cervical intraepithelial neoplasia; HPV: Human papillomavirus; HSIL: High-grade squamous intraepithelial lesion; LSIL: Low-grade squamous intraepithelial lesion; SCC: Squamous cell carcinoma.

CIN1 and CIN2 were combined into a single group due to their similar clinical management strategies, whereas CIN3 was retained as a separate group, reflecting severe dysplasia or carcinoma *in situ*. All clinical examinations were conducted in strict accordance with the World Medical Association Declaration of Helsinki, and written informed consent was obtained from all participants before enrollment.

The authors affirm the accuracy and completeness of the data and analyses, as well as adherence to all technical and bioinformatic protocols throughout the study. Notably, this investigation builds upon our prior research,[19,22] which established the specificity of the N-NOSE test in differentiating healthy individuals from cancer patients, with reported values of 90% and 95%, respectively. The present study employed the same cutoff values as those used in previous clinical investigations.[19,22]

For the cervical cancer cohort, tumor staging was conducted according to the Union for International Cancer Control Tumor-Node-Metastasis (TNM) classification system.[26] Consistent with standard clinical practices, staging included a combination of physical examinations, imaging modalities such as computed tomography or magnetic resonance imaging, and pathology results, where applicable, to evaluate tumor extent (T), nodal involvement (N), and metastasis (M). The final overall stage (I – IV) was assigned based on these combined findings. The detailed distribution – stage I (36 patients), stage II (14 patients), stage III (7 patients), and stage IV (8 patients) – was previously reported in our prospective clinical study,[24] which also provides comprehensive information on patient demographics, tumor characteristics, and methodological details. As CIN represents a pre-invasive process rather than an invasive malignancy, TNM staging was not applicable to the 245 patients with CIN. Instead, these pre-invasive lesions were classified based on cervical cytology findings in accordance with the Bethesda System 2001.[25] Cervical cancer screening procedures, including cytology and, when clinically indicated, colposcopy or histopathological examinations, were performed to confirm whether the patient had invasive cervical cancer, which requires TNM staging, or CIN.[24]

2.2. Culture and maintenance of *C. elegans*

C. elegans (wild-type N2) were cultured at 20°C on nematode growth media seeded with *Escherichia coli* (*E. coli*) strain NA22 as a food source, following standard protocols. *C. elegans*, a nematode approximately 1 mm long, reaches adulthood within 3 – 4 days and can lay 100 – 300 eggs. These nematodes were maintained under controlled conditions to ensure consistent and reproducible experimental results. The cultivation process adhered to established protocols to maintain the health and viability of the *C. elegans* populations throughout the study.

2.3. Measurement method of N-NOSE

Chemotaxis analysis of nematodes was conducted according to standard protocols used in previous nematode studies.[19,24,27] *C. elegans* was cultured at 20°C under well-fed and uncrowded conditions, with the *E. coli* strain NA22 as a food source.

Chemotaxis assays were conducted on 9 cm plates containing 10 mL of 2% agar supplemented with 5 mM potassium phosphate (prepared by combining appropriate amounts of monobasic potassium phosphate and dipotassium phosphate in ultrapure water), 1 mM calcium chloride, and 1 mM magnesium sulfate.[24] Briefly, 0.5 µL of 1 M sodium azide – an anesthetic used to minimize the

effects of adaptation – was spotted at four locations on the plate (for the urine side and non-urine side). Subsequently, 1 μL of urine sample, diluted 10-fold and 100-fold with ultrapure water, was added to two spots on the urine side. We confirmed that nematodes showed no chemotaxis behavior to 1 μL of water (data not shown).

Approximately 100 adult nematodes were collected and washed three times using chemotaxis buffer – prepared using 0.05% gelatin, 5 mM potassium phosphate (made by combining appropriate amounts of monobasic potassium phosphate and dipotassium phosphate in ultrapure water), 1 mM calcium chloride, and 1 mM magnesium sulfate. The nematodes were then placed at the center of the plate. After removing the excess buffer, the nematodes were allowed to roam freely for 30 min.

The chemotaxis index was calculated[19,24,28] using the following equation:

$$\text{Chemotaxis index} = \frac{(A - B)}{(A + B)} \quad (1)$$

Where (A) is the number of nematodes near the urine samples, and (B) is the number of nematodes in the region without the urine samples.

For each sample, chemotaxis assays were performed using both 10-fold and 100-fold urine dilutions (n = 10 for each dilution). A negative chemotaxis index (ranging from −1 to 0) indicates repulsion to the sample, while a positive chemotaxis index (ranging from 0 to 1) indicates attraction to the sample.[19,24] A chemotaxis index was considered "positive" if it was positive in at least one of the two dilutions.[22,24]

2.4. Statistical analyses

One-way analysis of variance was used to calculate the correlation coefficients and/or p-values between the N-NOSE index at both 10-fold and 100-fold urine dilutions and variables such as age and each biochemical blood test (hepatic alanine transaminase, hepatic aspartate aminotransferase, renal blood urea nitrogen, or renal creatinine). Statistically significant differences in the N-NOSE index for both urine dilutions (10-fold and 100-fold) between cervical cancer patients and CIN cases were evaluated using Welch's t-test. The Wilcoxon signed-rank test was employed to calculate the p-values between the N-NOSE index at both urine dilutions (10-fold and 100-fold) and each urine general qualitative test (urinary glucose, urinary ketone bodies, urinary proteins, or urinary occult blood), respectively. All statistical analyses were performed using JMP® 14 software (SAS Institute, USA). A $p<0.05$ was considered statistically significant.

3. Results

3.1. Sensitivity of N-NOSE in detecting cervical cancer

Early identification of cervical carcinogenesis is crucial for reducing the mortality risk associated with cervical cancer.[2,29] Therefore, our investigation focused on evaluating the capability of our N-NOSE technology to detect CIN, an early-stage pre-cursor to cervical cancer, with high sensitivity. While the absence of a concurrent control group is a limitation, the specificity of the N-NOSE test has been previously established in large-scale studies involving healthy individuals, demonstrating high specificity rates of 90% and 95% using the same cutoff values and methodologies applied in this study.[19,22] This methodological consistency allows us to infer the specificity of the test within our study population. Our primary objective was to assess the test's ability to identify true positive cases within a diseased cohort correctly. The high sensitivity observed, particularly the 100% sensitivity for detecting CIN3 lesions, underscores the test's potential clinical utility in the early detection and intervention of cervical carcinogenesis. Early identification of CIN is critical to prevent the progression of invasive cervical cancer, and our findings support the role of N-NOSE as a non-invasive and sensitive screening tool.

In this study, we employed the same cutoff values as those used in our previous clinical investigations, as illustrated in Figure 2, to ensure consistency and comparability. This methodological alignment allows us to infer specificity based on established data while focusing on evaluating sensitivity within the present patient cohort. A "negative" result is defined as nematode repulsion to both 10- and 100-fold diluted urine samples, while a "positive" result indicates nematode attraction to at least one of the diluted urine samples. In our previous studies, specificity rates of 90% and 95% were confirmed using the same cutoff values in two independent populations of healthy individuals.[19,22] Conducting the N-NOSE chemotaxis assays under the same conditions as we did in our previous studies allows us to maintain consistency and comparability with our earlier findings while acknowledging the inherent constraints posed by the study design.

The test was conducted on urine samples from 74 female patients with cervical cancer and 245 female patients with CIN. Statistical analysis revealed no significant correlation between the results of N-NOSE and age, with correlation coefficients of -0.04166 for 10-fold dilution (p=0.4585) and -0.00517 for 100-fold dilution (p=0.9267). Among the cervical cancer patients, 53 out of 74 patients (71.6%) tested positive using the N-NOSE test. Stage-specific sensitivities of cervical cancer using the N-NOSE test, based on the

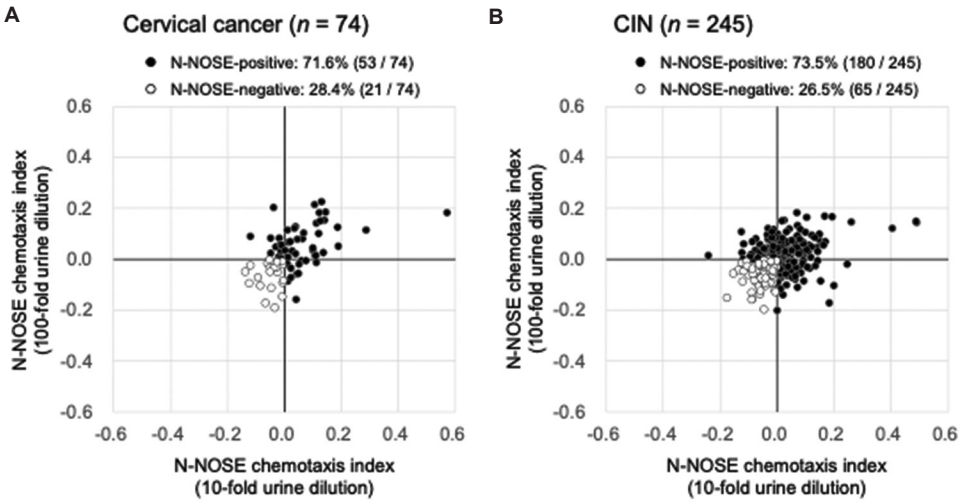

Figure 2. N-NOSE chemotaxis assay results in cervical cancer (A) and CIN (B) patients. N-NOSE chemotaxis assays were conducted using urine samples from 74 cervical cancer patients and 245 patients with CIN. Each sample was tested at 10-fold and 100-fold dilutions (n=10/dilution), and the chemotaxis indices were averaged. Positive N-NOSE result was defined by a chemotaxis index between 0 and 1 in at least one dilution. Closed circles represent N-NOSE-positive, while open circles indicate N-NOSE-negative.
Abbreviations: CIN: Cervical intraepithelial neoplasia; N-NOSE: Nematode-NOSE.

74 cases enrolled in this study, were previously reported in our publication[23]: Stage 0 (85.7%), stage I (83.3%), stage II (57.1%), stage III (71.4%), and stage IV (50.0%). Similarly, 180 out of 245 (73.5%) CIN patients demonstrated positive N-NOSE results, as illustrated in Figure 2 and Table 1. In Table 1, CIN3 was presented separately (n = 9) to emphasize its classification as severe dysplasia or carcinoma *in situ*, which carries a higher risk of progression to invasive cancer. In contrast, CIN1 (mild dysplasia) and CIN2 (moderate dysplasia) (n = 236) were grouped together due to their shared clinical management protocols and less advanced pathologies. This grouping aligns with our study design, in which all CIN cases were classified according to the Bethesda System 2001 guidelines as CIN1, CIN2, and CIN3.

Notably, all nine patients with CIN3 exhibited positive N-NOSE results (Table 1). Statistical analysis revealed no significant differences in N-NOSE results between cervical cancer and CIN patient groups, as illustrated in Figure 2, with p-values of 0.2520 for 10-fold dilution and 0.1072 for 100-fold dilution. These findings suggest that N-NOSE may be capable of detecting early cervical carcinogenesis, such as CIN, with a similar sensitivity as it does for invasive cervical cancer.

3.2. Correlations between N-NOSE results and biochemical makers

Biochemical blood tests revealed no significant correlations between the N-NOSE results and various markers of liver and kidney function, including hepatic alanine transaminase (10-fold: p=0.5466, 100-fold: p=0.6492), hepatic aspartate aminotransferase (10-fold: p=0.3190, 100-fold: p=0.6866), renal blood urea nitrogen (10-fold: p=0.5180, 100-fold: p=0.1154), and renal creatinine (10-fold: p=0.5474, 100-fold: p=0.7973). In contrast, general qualitative urine tests demonstrated significant correlations between the N-NOSE results and both urinary glucose (graded as 4+, indicating concentrations ≥1,000 mg/dL) (10-fold: p=0.0968, 100-fold: p=0.0355) and urinary ketone bodies (10-fold: p<0.0001, 100-fold: p=0.0235). However, no significant correlations were observed with urinary proteins (10-fold: p=0.2025, 100-fold: p=0.5294) or urinary occult blood (10-fold: p=0.9137, 100-fold: p=0.7385).

Table 1. N-NOSE sensitivity for cervical cancer and CIN patients

Cervical carcinogenesis	Number of patients	N-NOSE positive	Sensitivity (%)
Cervical cancer	74	53	71.6
CIN	245	180	73.5
CIN1/2	236	171	72.5
CIN3	9	9	100.0

Abbreviations: CIN: Cervical intraepithelial neoplasia; N-NOSE: Nematode-NOSE.

4. Discussion

The WHO advocates for a comprehensive approach to control cervical cancer, which includes primary prevention (HPV vaccination), secondary prevention (screening and treatment of pre-cancerous lesions such as CIN), tertiary prevention (diagnosis and treatment of

invasive cervical cancer), and palliative care.[1] Although high-income countries have significantly reduced cervical cancer mortality through robust prevention and treatment programs, low- and middle-income countries often lack access to these resources. This disparity contributes to a disproportionately higher disease burden in these countries, highlighting the urgent need for improved global access to preventive and therapeutic measures.[1,9,30]

The curability of cervical cancer, when diagnosed at an early stage, underscores the urgent need for highly sensitive, affordable, and non-invasive screening methods that can detect CIN before it progresses to invasive cervical cancer. Our study demonstrates the potential of the N-NOSE test – a *C. elegans* scent-based test using urine – as a highly sensitive tool for detecting CIN and, potentially, early-stage cervical cancer. Notably, previous studies have demonstrated that N-NOSE is also effective in the early detection of various other cancers, including biliary tract, gallbladder,[23] esophageal,[31] pancreatic,[32,33] and gastric cancers.[23] The ability of N-NOSE in identifying early-stage cancer across different types may be attributed to the nematode's highly evolved sense of smell.[34,35]

The N-NOSE test leverages the chemotaxis of *C. elegans* in response to cancer-related VOCs[21,36,37] and has demonstrated higher sensitivity for early-stage cancer detection compared to conventional tumor biomarkers such as CEA or CA19-9. This sensitivity may be linked to early metabolic changes in cancer cells, such as the Warburg effect (aerobic glycolysis),[38,39] which often precedes or accompanies the epithelial-to-mesenchymal transition. In addition, cancer cells exhibit upregulation of other metabolic pathways such as glutaminolysis, fatty acid synthesis, the pentose phosphate pathway, and mitochondrial fatty acid oxidation.[38,39] Further investigation into the specific VOCs detected by *C. elegans* and their association with various types of cancer may contribute to the development of more targeted and effective cancer screening and diagnostic tools.

In this study, significant correlations were observed between the N-NOSE results and specific urinary biomarkers, including glucose (graded as 4+, indicating concentrations ≥1,000 mg/dL) and ketone bodies. While elevated glucose levels were observed in a small subset of patients (4/319), potentially influencing test results, a previous study[22] suggests that this factor does not significantly impact the overall N-NOSE accuracy. Similarly, significant correlations were observed between the N-NOSE results and urinary ketone bodies in 16 out of 319 patients, encompassing both CIN and cervical cancer cases. This finding aligns with the understanding that cancer progression can alter energy metabolism, leading to increased ketone body production. Certain ketone bodies are known to attract nematodes,[28,40] and their presence in urine may explain the observed chemotaxis. Further research is needed to determine the precise relationship between urinary ketone body concentrations and chemotaxis responses of nematode.

Despite the promising results demonstrated by N-NOSE, our study has several limitations that should be addressed. One of the limitations is the lack of a control group (i.e., healthy subjects or non-cancerous individuals) to evaluate N-NOSE specificity. However, this study focuses on evaluating the sensitivity of the N-NOSE test in detecting CIN and cervical cancer among diagnosed patients. While the absence of a concurrent control group is a constraint, it is important to note that the specificity of the N-NOSE test has been thoroughly established in previous large-scale studies involving healthy individuals. These studies demonstrated high specificity rates of 90% and 95% using the same cutoff values and methodologies employed in our research,[19,22] allowing us to reasonably infer the specificity of the test within our study population.

The N-NOSE test operates based on the chemotaxis response of *C. elegans*, where nematodes are attracted to urine samples from cancer patients (positive chemotaxis index) and repelled by urine samples from subjects without cancer (negative chemotaxis index).[19] The consistent cutoff value (0) used in this study is similar to those used in previous N-NOSE clinical studies,[19,22] reinforcing the relevance of our study. While high specificity is essential to avoid unnecessary interventions, the balance between sensitivity and specificity is crucial for effective screening.[41-43] In this regard, N-NOSE emerges as a promising screening tool, demonstrating superior sensitivity across various cancers, particularly for detecting early-stage cancers.

Our primary objective was to accurately assess the test's ability to identify true positive cases within a diseased cohort. The high sensitivity observed – particularly the 100% sensitivity in detecting CIN3 lesions – highlights the test's potential clinical application for early detection and intervention. Early identification of CIN is crucial for preventing its progression to invasive cervical cancer. The findings in this study support the N-NOSE test as a non-invasive, sensitive screening tool with the potential to improve patient outcomes.

Translating these prospective findings into real-world clinical applications requires careful consideration of the inherent challenges associated with detecting disease in asymptomatic individuals. In these cases, the low signal-to-noise ratio can lead to an overestimation of sensitivity in studies where cases are pre-identified.[41-43] Real-world screening programs often encounter variability in

diagnostic test accuracy, impacting the performance of the screening test.[44-46] This discrepancy may arise due to prospective analyses inadvertently favoring exceptionally high diagnostic accuracy scenarios that do not reflect the variability encountered in routine clinical settings.[42,47,48]

Even with limitations in specificity and sensitivity, the use of cancer screening tests in asymptomatic populations can significantly reduce cancer-related mortality.[49,50] Early detection of cancer enables timely intervention and improves survival rates, although outcomes may still be affected by aggressive cancer types and other factors beyond diagnosis.[41,50] Reducing the incidence of late-stage diagnoses offers substantial benefits for both patients and healthcare systems.[51] To maximize the impact of screening, it is essential to adopt personalized screening intervals, continuously develop and improve the tests, and integrate them into comprehensive prevention and treatment strategies. Even moderately performing tests can contribute to public health outcomes by enhancing accessibility, affordability, and participation while driving technological advancements and promoting health awareness.[50,52]

Our findings suggest that the N-NOSE test is a highly sensitive, non-invasive, and convenient method suitable for detecting early cervical carcinogenesis, such as CIN. This aligns with the growing interest in novel, accessible screening tools that can address disparities in cervical cancer detection and prevention.[9,53] Further research is warranted to confirm these findings in diverse populations, particularly in underserved communities with limited access to traditional screening methods. Exploring the potential of N-NOSE as a tool for detecting early-stage cancer may offer a promising opportunity to advance global health equity in cervical cancer prevention.

5. Conclusion

In this study, N-NOSE has demonstrated the potential to identify CIN as a pre-cancerous stage, as well as subsequent intraepithelial lesions and invasive cervical cancer. Our N-NOSE test offers an affordable and non-invasive approach for the early detection of progressive cervical carcinogenesis, enabling timely intervention and treatment. This is particularly significant for low- and middle-income countries with limited access to traditional screening methods.

As highlighted in our recent review,[9] the development and implementation of innovative, accessible screening tools, such as N-NOSE are crucial for addressing global disparities in cervical cancer detection and prevention. The commercial availability of N-NOSE in Japan, with over 700,000 individuals screened to date, underscores its potential to improve public health.

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Conflict of interest

The authors are employees of HIROTSU BIO SCIENCE Inc., which developed and commercialized the N-NOSE technology described in this paper. T.H. is the CEO and Founder. The authors declare that these affiliations have not influenced the design, conduct, interpretation, or reporting of the research presented in this study.

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Project administration: Takaaki Hirotsu
Writing – original draft: Hideyuki Hatakeyama
Writing – review & editing: Hideyuki Hatakeyama, Aya Hasan Alshammari, Masayo Morishita, Umbhorn Ungkulpasvich, Junichi Yamaguchi, Eric di Luccio

Ethics approval and consent to participate

This prospective study was conducted in accordance with the Declaration of Helsinki and received ethical approval from the Ethics Committee of the National Hospital Organization Shikoku Cancer Center (approval number: 3045-1). All participants provided written informed consent before enrollment, which included an agreement for the collection and analysis of urine samples for research purposes, as well as the potential publication of anonymized data. The study did not involve any interventions or changes to the participants' standard clinical care.

Consent for publication

Written informed consent for publication was obtained from all participants involved in the study.

Availability of data

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

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ACCSCIENCE PUBLISHING

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ORIGINAL ARTICLE

Maternal and neonatal outcomes in placenta previa complicated by anterpartum hemorrhage

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Abstract

Background: Antepartum hemorrhage (APH) during pregnancy has a significant impact on both mothers and neonates. **Aim:** This study aims to investigate maternal and neonatal outcomes by retrospectively analyzing placenta previa (PP) cases complicated by APH. **Methods**: This study retrospectively investigated 50 cases of singleton pregnancies in women diagnosed with PP managed at a tertiary care Hospital in Karachi, Pakistan, from January to October 2024. The participants recruited were of a gestation period ranging from 24 to 42 weeks. The participants were categorized into two groups: APH and non-APH. The study assessed various maternal clinical characteristics. Ultrasound exams were conducted using standardized methods. Perinatal outcomes for mothers and newborns were compared, including complications such as preterm delivery, placental abruption, and neonatal issues. Mann–Whitney *U* test, Student's *t*-test, Chi-square test, and binary logistic regression were employed for statistical analysis. **Results:** Women afflicted with APH exhibited significantly higher maternal age compared to those without APH (p=0.029). Furthermore, we discovered a notable statistical difference in the type of PP between the two groups. Specifically, complete PP was observed in 27.3% of women with APH but only in 4.3% of those without APH. The gestational age at delivery for pregnant women with APH was notably shorter compared to those without APH, leading to a considerably higher incidence of both lower birth weights and preterm deliveries when APH was present, in contrast to cases where APH was absent. **Conclusion:** APH has a significant association with maternal and neonatal outcomes, especially in cases of PP. Thus, timely interventions are necessary to prevent APH to mitigate further complications in mothers and newborns. **Relevance for Patients:** The research demonstrates why prompt diagnosis and on-time care interventions improve maternal–fetal health outcomes during APH incidents, specifically in PP patients.

Keywords: Antepartum hemorrhage; Pregnancy; Placenta previa; Perinatal outcomes

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1. Introduction

The term "placenta previa" (PP) is defined as the partial or complete obstruction of the internal os of the cervix that occurs during pregnancy when the placenta is situated low in the uterus. The overall incidence of this disorder ranges between 0.3% and 2% during

the third trimester of pregnancy. However, variations in the incidence rate of PP have been reported in certain regions including Pakistan.[1]

There are four types of PP: Low-lying, marginal previa, partial previa, and full previa.[2] Vaginal bleeding occurring in the second half of pregnancy (after 20 weeks of gestation and before the onset of labor) is known as antepartum hemorrhage (APH), which is the global leading cause of maternal morbidity, with PP being one of the underlying causes.[3] Research has shown a significant link of very early APH to the occurrence of intrapartum hemorrhage, leading to cesarean delivery.[4] For example, some women experience life-threatening APH, necessitating not only an early cesarean section but also a hysterectomy. In contrast, in other cases, the woman may elect a planned term cesarean section for no other reason than constitutional vagaries. APH is typically induced by both PP and placental abruption, although the precise etiology remains uncertain.[5]

PP is at the top of the list of the most unfortunate abnormalities in pregnancy, causing various maternal and neonatal complications. These outcomes are most commonly attributed to maternal antepartum and intrapartum hemorrhage. A study has reported that the prevalence of PP in women with APH was ten times greater than that in women without PP.[6] The rapid increase in cesarean birth rates has led to a concomitant upsurge in the prevalence of PP, so much so that PP is now recognized as a significant global public health problem.[7,8] APH is commonly seen in pregnant women with PP but has received little attention in certain areas. According to several studies, as well as the World Health Organization (WHO) definition, pregnant women with PP are at an increased risk of APH than those without.[4,9] The prevalence rate of PP reported by research studies varies in a range of 20 – 90%.[10,11] The incidence of previa is also predicated on maternal age, position of previa (anterior or posterior), type of previa (complete or incomplete), population characteristics, lifestyle habits, and diagnostic standards used.[12-15]

This retrospective case–control investigation evaluated maternal and newborn results using data from pregnant women who did have pregnancy proteinuria alongside those who did not have APH to find clinical markers which could help in the early diagnosis of APH and preventive strategies.

2. Materials and methods

2.1. Study population

In this retrospective study, we examined 56 cases of singleton pregnancies in women who were diagnosed with PP and subsequently delivered at a tertiary care hospital in Karachi, Pakistan, from January to October 2024. These participants were of a gestational age ranging from 24 to 42 weeks. The study was approved by the Institutional Review Board of PNS Shifa Hospital Karachi. All subjects provided informed consent before being included in the study. All relevant protocols were adhered to. The participants were divided into two groups: APH and non-APH. The diagnosis of PP was confirmed using transabdominal and transvaginal ultrasounds conducted at admission, which were later validated at the time of delivery. In this study, APH was defined as intermittent or continuous vaginal bleeding leading to ≥20 mL, or total antepartum blood loss from the second half of pregnancy till delivery. Individuals who had more than one pregnancy, no prenatal treatment, a gestational age of >24 weeks, or no access to medical data were excluded. Additional exclusion criteria also include women bearing fetuses with abnormalities, individuals with incomplete antenatal or outcome data, and women suffering vaginal bleeding with total antepartum blood loss not exceeding 20 mL. It is demanding to set apart the blood with tinged mucus or minute quantities of blood, which is why women with vaginal bleeding but total antepartum blood loss of <20 mL were not included.

We assessed various clinical characteristics of the mother, including the age, gestational age at delivery, parity, gestational diabetes mellitus, pre-gestational diabetes mellitus, obesity, prior cesarean delivery, preeclampsia, *in vitro* fertilization, antepartum uterine contractions, and prior dilatation and curettage. The WHO classified pre-pregnancy obesity as having a body mass index (BMI) of >25 kg/m^2 for Asian populations. Three or more uterine contractions per 30 min between 24 and 34 weeks were considered antepartum uterine contractions.[16] Adherent placenta is a condition determined with the presence of ultrasound markers for placenta accreta spectrum disorder and sonographic features such as retroplacental clear space depletion, ≥4 lacunar spaces, cervix jellyfish sign, increased blood supply to the non-uniform bladder wall, and reduced myometrial thickness (<1 mm diameter).[16]

2.2. Ultrasound examination

In each case, transvaginal and transabdominal ultrasonography was used for assessment. According to the new diagnostic criteria, the internal cervical os is covered, either completely or partially, by the placenta in women with PP. Standardized methods were used to conduct cervical exams. We acquired a sagittal plane to view the cervix in its entirety. Three cervical length measurements were made, with the shortest measurement being noted, and <2.5 cm length was considered a short cervical length.[13,16]

2.3. Perinatal outcomes

The outcomes for mothers and newborns in both APH and non-APH groups were compared. Maternal pregnancy outcome variables included preterm delivery, placental abruption, cesarean hysterectomy, emergency cesarean section, bladder damage, postpartum hemorrhage, and postoperative admissions to the intensive care units (ICUs). A composite unfavorable pregnancy outcome was defined as the existence of one or more of these complications.

Neonatal complications were assessed using variables such as neonatal death, preterm birth, admission to the neonatal ICU, Apgar score at 5 min after birth, intraventricular hemorrhage, respiratory distress syndrome, and infection. A living newborn that passes away within 28 days of delivery is referred to as a neonatal death. The composite unfavorable neonatal outcome included one or more of the abovementioned issues.[12]

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 27 (IBM Corporation, USA). The normality of the data was assessed by using various statistical tests, including the Shapiro–Wilk test. The Mann–Whitney U test was used to compare the continuous variables. Pearson Chi-square test was used to compare categorical variables. The odds ratios (OR) for the categorical variables were calculated using the Pearson Chi-square test, and univariate logistic regression was utilized to analyze ordinal and continuous variables. Lastly, independent risk factors were identified using multivariate logistic regression. The threshold was set at $p<0.05$.

3. Results

Women in the APH group exhibited significantly higher maternal age than those in the non-APH group (30.6 ± 2.7 vs. 28.4 ± 4.1, OR = 1.221 [1.45 – 1.03], $p=0.03$). The type of PP was also significantly different between the two groups ($p=0.03$), with complete PP present in 27.3% of women with APH but in only 4.3% of those without APH. APH was correlated with markedly increased blood loss (487.2 ± 213.6 vs. 253.5 ± 142.3, OR = 1.01 [1.01 – 1.00], $p<0.0001$) and greater demand for intraoperative blood product transfusion (348.5 ± 318.3 versus 23.8 ± 109.1, OR = 1.01 [1.01 – 1.00], $p<0.0001$). Women with APH also had significantly lower hemoglobin after delivery than women without APH (10.2 ± 1.1 vs. 10.9 ± 0.87, OR = 0.48 [0.88 – 0.26], $p=0.01$). The same is depicted in Table 1.

While there was no statistically significant difference in placental location, the most common position in the APH group was anterior implantation (50% in the APH group versus 42.3% in non-APH group). The proportion of women having posterior placental placement was significantly higher in the non-APH group (65.2%) compared to the APH group (34.4%). Though not statistically significant, a higher percentage of women in the APH group were graduates (57.6%) compared to the non-APH group (34.8%). The same is depicted in Table 2.

3.1. Maternal outcomes

More than half of the women in the APH group (57.6%) had undergone emergency cesarean sections compared to 4.3% in the non-APH group (OR = 29.86 [248.64 – 3.59], $p<0.0001$). Women with APH delivered at a significantly earlier gestational age than those without APH (35.5 ± 2.3 versus 37.5 ± 1.0, OR = 0.35 [0.65 – 0.19], $p<0.0001$), resulting in a significantly higher occurrence rate of preterm delivery among women with APH compared to their non-APH counterparts (60.6% versus 4.3%, OR = 13.33 [54.77 – 3.25], $p<0.0001$). Our results in Table 3 also showed that APH instigated cesarean hysterectomy in over 10% of the women, in contrast to 0 in women without APH.

3.2. Neonatal outcomes

Babies born to patients with APH had significantly lower birth weights (2570 ± 531.8 versus 2945.5 ± 444.8, OR = 0.998 [1.00 – 0.997], $p=0.002$) and were mostly preterm (66.7% vs. 8.7%, OR = 21.00 [106.22 – 4.15], $p<0.0001$), compared to those born to non-APH patients. There was also a significantly increased incidence of neonatal ICU admission (24.2% versus 4.3%, OR = 7.04 [60.82 – 0.82], $p=0.046$) and respiratory distress syndrome in the APH group (27.3% vs. 4.3%, OR = 8.25 [70.50 – 0.97], $p=0.028$). Neonatal outcomes with 5% higher frequency in the APH group than the non-APH group include neonatal death, small-for-gestational-age, Apgar score at 5 min <7, and sepsis. The same is shown in Table 4 below.

Multivariate logistic regression analysis was conducted for all variables that were significant in the univariate analysis, including age, gestational age at delivery, delivery method, type of PP, estimated intraoperative blood loss, intraoperative blood transfusion, birth weight, hemoglogin after delivery, preterm delivery, emergency cesarean section, preterm birth, neonatal ICU admission, and respiratory distress syndrome. However, none of the risk factors turned out to be significant in the analysis.

4. Discussion

This study is a distinctive case–control retrospective study conducted at a tertiary care hospital in Karachi, Pakistan. The study includes a sample of 50 pregnant women with PP, comprising both individuals with and without APH. APH with an underlying cause of PP occurs due to the

Table 1. Demographic characteristics and ultrasound features of the study participants

Variables	APH groups (*n*=33)	Non-APH group (*n*=23)	*p*-value	OR (95% CI)
Maternal age[a]	30.6±2.7	28.4±4.1	0.03	1.22 (1.45 – 1.03)
Gestational age at delivery[b]	35.5±2.3	37.5±1.0	<0.0001	0.35 (0.65 – 0.19)
Parity (number of live births)[b]	2.3±1.4	1.61±1.3	0.06	1.51 (2.29 – 1.00)
Gravidity (total number of pregnancies)[b]	3.6±2.0	2.8±2.2	0.15	1.29 (1.77 – 0.93)
Number of artificial abortions[b]	0.52±0.71	0.48±0.1	0.34	1.06 (2.03 – 0.55)
Delivery method[c]		(n=22)	<0.0001	
C-section	4 (12.1%)	9 (40.9%)		
Vaginal	6 (18.2%)	12 (54.5%)		1.13 (5.21 – 0.24)
Emergency C-section	23 (69.7%)	1 (4.5%)		51.75 (528.10 – 5.07)
Estimated intraoperative blood loss (mL)[b]	487.2±213.6 (n=32)	253.5±142.3 (n=20)	<0.0001	1.01 (1.01 – 1.00)
Intraoperative blood transfusion (mL)[b]	348.5±318.3	23.8±109.1 (n=21)	<0.0001	1.01 (1.01 – 1.00)
Previous cesarean delivery[b]	1.1±1.1	1.0±1.3 (n=21)	0.33	1.17 (1.88 – 0.73)
Type of PP[c]			0.03	
Marginal PP	7 (21.2%)	11 (47.8%)		
Partial PP	16 (48.5%)	10 (43.5%)		2.51 (8.64 – 0.73)
Complete PP	9 (27.3%)	1 (4.3%)		14.14 (137.30 – 1.46)
Low-lying placenta	1 (3.0%)	1 (4.3%)		-
Placental location[c]	(n=32)		0.07	
Anterior	16 (50.0%)	7 (30.4%)		
Posterior	11 (34.4%)	15 (65.2%)		0.46 (4.67 – 0.05)
Lateral	5 (15.6%)	1 (4.3%)		0.15 (1.44 – 0.02)
Birth weight (g)[b]	2570±531.8 (n=30)	2945.5±444.8 (n=22)	0.002	0.998 (1.00 – 0.997)
Hemoglobin before delivery[b]	11.4±1.1 (n=32)	11.6±0.9	0.58	0.83 (1.45 – 0.47)
Hemoglobin after delivery[a]	10.2±1.1 (n=32)	10.9±0.9	0.01	0.48 (0.88 – 0.26)
Education level[c]			0.21	
High school or less	8 (24.2%)	7 (30.4%)		
College	6 (18.2%)	8 (34.8%)		0.66 (2.84 – 0.15)
Graduate	19 (57.6%)	8 (34.8%)		2.08 (7.69 – 0.56)

Notes: [a]Independent *t*-test, [b]Mann–Whitney U test, [c]Pearson Chi-square test; Values are expressed as either mean±SD or count (percentage).
Abbreviations: APH: Antepartum hemorrhage; CI: Confidence interval; OR: Odds ratio; PP: Placenta previa.

mechanical stretch between the placenta and uterus, leading to bleeding that can complicate the pregnancy and thus undesirable outcomes. The uterine stretch at the placental site continues to increase in the latter half of pregnancy, thus posing a risk of rupture of the maternal blood vessels that can cause hemorrhage.[17] Furthermore, uterine contractions, premature cervical changes, and the type of PP can influence the likelihood of hemorrhage. Patients with complete PP are associated with a higher risk of bleeding and more complications as compared to other types because in this condition, the entire cervical os of the affected patients is covered.[1,13,17]

The aberrant attachment of PP predisposes it to vascular compromise and mechanical stress, increasing the likelihood of bleeding during pregnancy. Attachment to the lower segment of the uterus, where the thickness of myometrium is significantly lower, compromises the stable environment where the placenta grows, and additionally, as the pregnancy progresses, there is a substantial expansion in the lower segment, which leads to increased stress.[16] This disrupts the decidua basalis – a thin and poorly vascularized membrane susceptible to detachment from mechanical stress – which plays a crucial role in placental attachment, thereby compromising the maternal blood vessels in intervillous space. Furthermore, cervical dilation and uterine contractions augment the risk of vascular compromise. Separately, higher fibrin deposition, maternal malperfusion, and chronic hypoxia

Table 2. Maternal and obstetric risk factors for APH in placenta previa cases

Variables	APH group (*n*=33) (%)	Non-APH group (*n*=23) (%)	*p*-value	OR (95% CI)
In vitro fertilization[a]	1 (3.0)	0	0.40	-
Obesity[a]*	1 (3.0)	0	0.40	-
Gestational diabetes mellitus[a]	7 (21.2)	1 (4.3)	0.08	5.92 (51.92 – 0.68)
Pre-gestational diabetes mellitus[a]	2 (6.1)	0	0.23	-
Preeclampsiaa	6 (18.2)	1 (4.3)	0.12	4.89 (43.71 – 0.55)
Hypertension before pregnancy[a]	5 (15.2)	1 (4.3)	0.20	3.93 (36.12 – 0.43)
Prior dilatation and curettage[a]	6 (18.2)	2 (8.7)	0.32	2.33 (12.76 – 0.43)
Prior history of PP in previous pregnancies[a]	2 (6.1)	1 (4.3)	0.78	1.42 (16.64 – 0.12)
Antepartum uterine contractions[a]	0	1 (4.3)	0.23	-
Antepartum corticosteroid use[a]	7 (21.2)	6 (26.1)	0.67	0.76 (2.66 – 0.22)
34 – <37 weeks (preterm)	4 (57.1)	0		
≥37 weeks (term)	3 (42.9)	6 (100.0)		
Antepartum tocolytics use[a]	0	1 (4.3)	0.23	-
Short cervical length at admission (<2.5 cm)[a]	10 (30.3)	6 (26.1)	0.73	1.23 (4.05 – 0.38)
Adherent placenta[a]	2 (6.1)	0	0.23	-

Note: [a]Pearson Chi-square test, *Pre-pregnancy obesity is defined as a pre-pregnancy body mass index (BMI) of >25 kg/m^2; Values are expressed as count (percentage).
Abbreviations: APH: Antepartum hemorrhage; CI: Confidence interval; OR: Odds ratio; PP: Placenta previa.

Table 3. Maternal outcomes and risk factors in placenta previa with APH

Variables	APH group (*n*=33) (%)	Non-APH group (*n*=23) (%)	*p*-value	OR (95% CI)
Preterm delivery[a]	22 (66.7)	3 (13.0)	<0.0001	13.33 (54.77 – 3.25)
<34 weeks (extremely/very preterm)	4 (18.2)	0		
34 – <37 weeks (late preterm)	18 (81.8)	3 (100.0)		
Emergency cesarean section[a]	19 (57.6)	1 (4.3)	<0.0001	29.86 (248.64 – 3.59)
Placenta abruption[a]	3 (9.1)	0	0.14	-
Postpartum hemorrhage[a]	1 (3.0)	0	0.40	-
Cesarean hysterectomy[a]	4 (12.1)	0	0.08	-
Postoperative ICU admission[a]	1 (3.0)	0	0.40	-
Bladder injury[a]	2 (6.1)	0	0.23	-

Note: [a]Pearson Chi-square test; Values are expressed as count (percentage).
Abbreviations: APH: Antepartum hemorrhage; CI: Confidence interval; ICU: Intensive care unit; OR: Odds ratio.

Table 4. Neonatal outcomes and risk factors in placenta previa with APH

Variables	APH group (*n*=33) (%)	Non-APH (*n*=23) (%)	*p*-value	OR (95% CI)
Neonatal death[a]	2 (6.1)	0	0.23	-
Preterm birth[a]	22 (66.7)	2 (8.7)	<0.0001	21.00 (106.22 – 4.15)
Apgar score at 5 min <7[a]	2 (6.1)	0	0.23	-
Small for gestational age[a]	4 (12.1)	1 (4.3)	0.32	3.03 (29.09 – 0.32)
Neonatal ICU admission[a]	8 (24.2)	1 (4.3)	0.05	7.04 (60.82 – 0.82)
Respiratory distress syndrome[a]	9 (27.3)	1 (4.3)	0.03	8.25 (70.50 – 0.97)
Intraventricular hemorrhage[a]	1 (3.0)	0	0.40	-
Sepsis[a]	2 (6.1)	0	0.23	-

Note: [a]Pearson Chi-square test; Values are expressed as count (percentage).
Abbreviations: APH: Antepartum hemorrhage; CI: Confidence interval; ICU: Intensive care unit; OR: Odds ratio.

are common histopathological findings of PP. It can be attributed to increased villous agglutination, with a higher number of syncytial knots and hypoplasia observed in the distal villus.[18,19] In addition, the thinness of the lower segment limits the contraction ability of the uterine wall as it cannot stop bleeding from the ruptured vessels effectively, thus exacerbating bleeding in the case of APH. In a study that investigated pregnant women affected by PP, Im *et al.*[20] found that compared with those without APH, women who had APH suffered from increased antepartum contractions of the uterus (33.3% vs. 10.2%, p=0.002), short cervical length (<2.5 cm, 53.0% vs. 27.1%, p=0.003), lower placental weight (0.44 ± 0.11 vs. 0.49 ± 0.12 kg, p=0.03), and a higher rate of lesions due to villous agglutination (42.4% vs. 22.0%, p=0.01).[20]

There are multiple risk factors for APH in patients with PP. Scarring and fibrosis in the uterus lining can be a probable cause that can affect normal placental implantation. Moreover, multiparity can contribute to PP as well, since multiple pregnancies introduce changes in the normal uterus environment, affecting the normal placental implantation.[20] Additionally, maternal age, lifestyle factors, surgical history, and cervical length can complicate the condition. A maternal age of 35 years or more is associated with higher PP risk due to age-related morphological and vascular changes in the uterus, similar to our study in which maternal age (OR = 1.22 [1.45 – 1.03], p=0.03) was a significant factor for APH. Similar findings were reported by Im *et al.* (OR = 4.15 [1.51 – 11.49], p<0.01), Rosenberg *et al.* (OR = 1.08 [1.07 – 1.09], p<0.001), and Fan *et al.* (prevalence = 51.6%, 95% CI = 42.7 – 60.6%).[20-22] Lifestyle factors such as malnutrition and smoking can further exacerbate the condition. Surgical histories such as myomectomy or dilation and curettage further add to the risks. However, cervical length is found to be inversely associated with the risk of APH in patients with PP.[23,24] Thus, proper antenatal care is crucial in identifying such risk factors and preventing severe maternal and fetal outcomes.

The current study's findings indicate that APH is a notable concern for pregnant women with PP, and there is a large variation in its prevalence among different studies. The findings of our study suggest that pregnancies complicated by PP and APH have shown a greater vulnerability to adverse outcomes for both the mother and the infant. The APH group exhibited a higher incidence of emergency cesarean section and complications associated with hemorrhage. Furthermore, APH is strongly correlated with prematurity, as indicated by its connection with shorter gestational periods, higher chances of preterm labor, newborn complications (e.g., respiratory distress syndrome), hospitalization in the neonatal ICU, and lower birth weight. The present findings are consistent with the outcomes of other studies, confirming that preterm birth (p<0.0001, OR = 21.00 [106.22 – 4.15]), respiratory distress syndrome (p=0.03, OR = 8.25 [70.50 – 0.97]), and neonatal ICU admissions (p=0.05, OR = 7.04 [60.82 – 0.82]) contribute to a higher risk for APH.[10,15] Similarly, Im *et al.*[20] also reported that the risk for APH was significantly raised by preterm births (p=0.0001), lower birth weight (p<0.0001), and neonatal ICU admission (p=0.0001). Similar findings have also been reported by Rosenberg *et al.* (p<0.001).[21]

Given the unpredictable outlook of PP with APH and the associated dangers for both the mother and the baby, these findings offer evidence to endorse the guideline put forth by the Society for Maternal–fetal Medicine, which recommends delivery rescheduling in the instances of bleeding or difficulties during pregnancy at the late preterm stage.[25] PP and placental abruption are the leading causes of APH. Other risk factors include minor bleeding in the sinus, battledore placenta, vasa previa, cervicitis, velamentous cord insertion, genital trauma, tumors, infections, and coagulation disorders.[10,26] Our study recognized blood loss (OR = 1.01 [1.01 – 1.00], p<0.0001) as a potential contributor to APH, which is concordant with Vergani *et al.*'s findings[10] that blood loss at delivery (p=0.09) is related to APH in patients with PP. PP is the primary cause of APH during the advanced stages of pregnancy. Women who are diagnosed with PP face a substantially elevated risk, approximately four times higher, of encountering vaginal bleeding in the second trimester of pregnancy.[21] In exceptional cases, some women may require an early cesarean surgery and hysterectomy due to a severe and potentially life-threatening hemorrhage, which poses surgical risks with long-term complications such as fertility and psychological issues. Moreover, APH is further associated with higher septicemia rates, involvement of more invasive procedures, and prolonged stays at the hospital.[27]

The prevalence of APH, due to PP, varies from region to region. According to a meta-analysis conducted by Fan *et al.*,[22] the pooled prevalence of APH in PP individuals was estimated to be 51.6% (95% CI: 45.8 – 61.0). However, individual studies report a wider range. Such differences can be attributed to the complexity of maternal, neonatal, and demographic factors. The calculated prevalence was the highest in Asia (53.4%, 95% CI: 42.7 – 60.6) and North America (53.2%, 95% CI: 34.1 – 72.2), followed by Europe (48.5%, 95% CI: 20.3 – 76.7) and Africa (33.8%, 95% CI: 22.3 – 45.3).[23] Demographic factor has a significant influence on the prevalence, followed by the healthcare

access and socioeconomic factors. The prevalence was the lowest in Africa despite the resource-limited settings and low socioeconomic status. The lower incidence can be attributed to the underreporting of the cases, small sample sizes, and low number of studies conducted. Moreover, it also been found that there is a negative correlation between the incidence of APH due to PP and years of reporting (correlation coefficient [r] = −0.01, 95% CI: −0.02 – 0.001, p=0.03). Furthermore, multiparous pregnancies had a significantly positive correlation with the incidence of APH due to PP (correlation coefficient [r] = 0.66, 95% CI: 0.08 to 1.24, p=0.03). Thus, these correlation results support the conjecture that previous pregnancy history plays a role in APH incidence and contributes to future outcomes. According to epidemiological data,[22] the average prevalence has shown gradual reduction over time. Although the leading causes responsible for the decreased APH occurrence were not analyzed, it is very likely that improvements in the national healthcare systems, diagnostic procedures, and obstetric practice collectively contribute to the case reduction. Robust healthcare systems help deliver safe, timely, and appropriate cesarean sections, ensuring optimal health results for women and newborns during emergency obstetrics.[28] A multidisciplinary approach taking into account maternal and fetal outcomes, strict monitoring, timely interventions, and patient education is required to effectively manage APH in patients with PP. Early detection of PP can be helpful in timely diagnosis and effective management of patients. This can be achieved by second-trimester ultrasounds to identify the placental location, followed by serial scans to monitor the condition with time. However, once a diagnosis is confirmed, the patient should be counseled about her condition and must be educated accordingly regarding the potential complications and the need for medical care if bleeding occurs.[29] Planning a delivery effectively is the cornerstone of managing such complicated cases; as for stable patients who do not have significant bleeding, an elective cesarean section can be scheduled between 36 and 37 weeks for optimal neonatal outcomes and avoidance of spontaneous labor. Severe bleeding cases can be managed by a prompt delivery subsequent to a cesarean section and a corticosteroid administration for ensuring fetal lung maturity.[23]

The advice incorporates early diagnosis followed by proper monitoring combined with planned C-section delivery to minimize the chances of complex gestational complications among pregnant women affected by PP. However, future research shall focus on unraveling the underlying mechanisms behind abnormal placental implantation and finding innovative management techniques for effective management. An in-depth analysis of predisposing factors and genetics should be conducted with the purpose of improving the current detection methods in terms of their efficacy, to mitigate the burden of mortality and morbidity in APH cases. In addition, perennial challenges such as resource constraints and lack of financial support, as well as the low rate of medical consultations and patients' follow-up, facing the developing countries are elevated to not only a clinical but also a socioeconomic issue. Furthermore, larger-scale epidemiological studies are needed in regions like Asia or Africa in order to have a clear understanding of the incidence and burden of APH among patients with PP. These recommended actions should be executed to aid in the introduction of strategies focused on maternal and fetal health intended to lower the burden of this multifactorial complication and both the maternal and fetal mortalities and morbidities in developing regions.

5. Conclusion

The current study demonstrates the significant impact of APH during pregnancy on maternal and neonatal outcomes. The results emphasize the need for vigilant monitoring and timely interventions in pregnant women with PP to address modifiable risk factors via antenatal care, patient education, and preventive strategies so as to mitigate the risk of APH and its associated adverse consequences for both mothers and newborns.

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Conflict of interest

The authors declare that they have no competing interests to declare.

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Ethics approval and consent to participate

This study was conducted in adherence to the ethical guidelines and principles outlined in the Declaration of Helsinki. The Institutional Review Board of Qatar Hospital Karachi reviewed and approved the study under strict guidelines. Informed consent was obtained from all patients before their inclusion in the study. Furthermore, the data collected from the patients was anonymized to address privacy and confidentiality.

Consent for publication

Verbal consent was obtained from the patients for releasing their data and/or images in this study.

Availability of data

Data are available from the corresponding author upon reasonable request.

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ACCSCIENCE PUBLISHING

Journal of Clinical and Translational Research

ORIGINAL ARTICLE

Clinical characteristics and outcomes of hospitalized coronavirus disease 2019 patients treated with remdesivir in Saudi Arabia: A 1-year retrospective study

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Abstract

Background: The recent pandemic caused by coronavirus disease 2019 (COVID-19) has resulted in increased deaths globally, and effective treatment is crucial as a form of disease management. **Aims:** This study was aimed to describe the epidemiological characteristics and outcomes of hospitalized COVID-19 patients who have been administered with remdesivir (RDV) at King Faisal Specialized Hospital in Saudi Arabia. **Methods:** Hospitalized COVID-19 adult patients who received RDV therapy (from January to December 2021) were included in this retrospective case series. Patients' demographics, medical history, presenting symptoms, and laboratory results were collected. Statistical analyses were performed using the Statistical Packages for the Social Sciences and data were presented based on their statistical variables. **Results:** A total of 210 patients (56.1% females, 43.9% males) with a mean ± standard deviation (SD) age of 57.43 ± 18.4 years were included in our analysis. Pneumonia was manifested in 52.4% of patients. The most common associated comorbidities were hypertension (54.76%) and diabetes (44.76%). From 180 participants that were vaccinated, 73 and 90 patients received two or three COVID-19 vaccine doses, respectively. The most frequent presenting symptoms were fever (80.1%) and cough (62.6%). About 48.1% and 10.48% of the cohort received RDV with dexamethasone or tocilizumab, respectively. Overall, 45.71% of the patients needed oxygen therapy during hospitalization, and 21 patients with pneumonia required mechanical ventilation for a mean ± SD of 15.15 ± 16.46 days with the mean ± SD length of the hospital stay which was 14.9 ± 46.29 days. Moreover, 41 patients were admitted to the intensive care unit for a mean ± SD stay of 10.5 ± 11.05 days, and 26 patients had died. **Conclusion:** Despite previous vaccination, a significant portion of hospitalized COVID-19 patients in our cohort required oxygen therapy and experienced longer periods of hospital stay, highlighting the seriousness of COVID-19 infection for some patients regardless of vaccination status. **Relevance for patients:** This study highlights RDV's role in reducing oxygen need and hospitalization duration, aiding better outcomes for COVID-19 patients.

Keywords: Coronavirus disease-2019; Remdesivir; Saudi Arabia; Vaccination; Intensive care unit

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1. Introduction

The coronavirus disease-2019 (COVID-19) which originated back in December 2019 at Wuhan, China, was caused by the novel pathogen severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2).[1] As of December 2023, the World Health Organization had reported over 772 million confirmed COVID-19 cases and 7 million deaths.[2]

Most individuals with COVID-19 experience mild-to-moderate symptoms, including fever, headache, body pain, sore throat, and loss of taste or smell, while some may also experience difficulty breathing and diarrhea. The infection may also progress to severe pneumonia, causing congestion, hypoxia, multi-organ failure, and death.[3,4]

Remdesivir (RDV), a nucleoside analog, is a broad-spectrum antiviral that inhibits RNA-dependent RNA polymerase. It was previously recommended to control Ebola, Middle-East Respiratory Syndrome Coronavirus, and SARS-CoV.[5]

RDV was granted emergency use authorization by the food and drug administration on May 01, 2020, for hospitalized COVID-19 patients and was officially approved on October 22, 2020.[6,7]

The adaptive COVID-19 treatment trial (ACTT) reported a significant reduction in recovery time and disease progression among hospitalized COVID-19 with pneumonia.[8] Furthermore, the final report from the SOLIDARITY trial emphasized that RDV usage resulted in reduced mortality among hospitalized COVID-19 patients on oxygen therapy but not mechanical ventilation.[9,10]

Several studies have reported a positive effect of RDV on COVID-19 clinical outcomes, including survival rates, clinical status, the need for mechanical ventilation, intensive care unit (ICU) admission, and hospitalization period with acceptable safety profile.[11-15] However, there is no past data related to the efficacy of RDV among the Saudi Arabia population. The current study aimed to describe the epidemiological, clinical, and outcomes of COVID-19 patients who received RDV and to investigate the length of their hospital stay and oxygen therapy administered in a specialized hospital in the Kingdom of Saudi Arabia (KSA).

2. Materials and methods

2.1. Study design, population, and treatment

This study was a retrospective case series of hospitalized COVID-19 patients admitted to the King Faisal Specialized Hospital in KSA between January and December 2021. COVID-19 patients ≥18 years of age verified with polymerase chain reaction using nasopharyngeal sample and administered with RDV were eligible for the study. RDV was administered intravenously for 5 days with a loading dose of 200 mg on day 1 and 100 mg on day 2 onwards.

2.2. Data collection

Patients' demographics and baseline characteristics including age, gender, body mass index (BMI), and smoking history were collected from the King Faisal Specialized Hospital in KSA database for medical records. Data on their medical history including the presence of hypertension, diabetes, cardiovascular disease, bronchial asthma, chronic obstructive pulmonary disease, chronic kidney disease, end-stage renal disease, active cancer, human immunodeficiency virus infection, solid organ transplant, bone marrow transplant, autoimmune disease, liver disease, cerebrovascular disease, chronic neurological or neuromuscular disease, and COVID-19 vaccination status were also collected. For COVID-19 vaccination status, the number of doses administered before COVID-19 diagnosis was also collected. In addition, each patient's most common presenting symptoms were reported. Laboratory parameters, including lymphocyte count, C-reactive protein (CRP) levels, and ferritin levels, were also included for the analysis, with ferritin levels categorized as <60 or ≥60 ng/mL.

Concomitant medications, vaccination status, laboratory findings, oxygen therapy, complications, and clinical outcomes, including the length of hospital stay, ICU admission, the need for mechanical ventilation, and death, were collected from the electronic health records available in the hospital database system.

2.3. Statistical analysis

Statistical analyses were performed using IBM Statistical Packages for the Social Sciences (SPSS) Statistics version 24 (SPSS, Chicago, IL, USA). The data were presented as numbers and frequencies for categorical variables and mean with standard deviations (SD) for continuous variables. The Chi-square (χ^2) test was used for categorical variables, while the *t*-test was used for continuous variables. The Kaplan–Meier curve was used to assess the probability of length of hospital stay associated with patients' gender, type of oxygen therapy received, and vaccination status.

3. Results

3.1. Patient characteristics

We have included 210 patients (56.1% females and 43.9% males) in our analysis, with a mean ± SD age of 57.43 ± 18.4 years. Of the 210 confirmed COVID-19 patients,

110 of them (52.4%) had pneumonia. The average BMI was 27.77 ± 8.84 kg/m^2. The most common comorbidities were hypertension (54.76%), diabetes mellitus (44.76%), and cardiovascular disease (30%). Here, 180 (85.7%) participants had COVID-19 vaccine. Nine (5%) patients were single-dose vaccinated, 73 (40.6%) patients received two doses of vaccine, 90 (50%) patients received three doses, and 6 (3.33%) patients received four doses before the diagnosis of COVID-19. However, the number of doses for 2 patients (1.11%) was not available. The most frequent presenting symptoms were fever (80.1%), cough (62.6%), and shortness of breath (41.1%). From the laboratory findings, the mean ± SD lymphocyte count was 3.79 ± 21.87 cells/μL, and the glomerular filtration rate was ≥60 in 56.2% of the patients, with no significant difference among patients with or without pneumonia. The mean ± SD CRP and ferritin were 3.79 ± 21.87 μg/mL and 106.4 ± 326.2 ng/mL, respectively (Table 1).

Regarding concomitant medications, 101 patients (48.1%) received RDV plus dexamethasone, and 22 (10.48%) received RDV with tocilizumab (Table 2).

3.2. Clinical outcomes

There were 96 (45.71%) of the included patients needed oxygen therapy during hospitalization, 86 (40.95%) received low-flow oxygen treatment through nasal cannula with a mean ± SD duration of 4.87 ± 5.42 days, and 22 (10.48%) received high-flow oxygen treatment through nasal cannula for a mean ± SD duration of 2.95 ± 4.37 days. Furthermore, 21 patients (10%) with pneumonia required mechanical ventilation for a mean ± SD duration of 15.15 ± 16.46 days (Table 3).

As for the complications, 14 patients (6.67%) suffered from bacterial pneumonia, 14 patients (6.67%) developed acute respiratory distress syndrome, and 9 patients (4.29%) suffered from septic shock. Overall, the mean ± SD length of the hospital stay for all patients was 14.9 ± 46.9 days. Forty-one patients (19.52%) were admitted to the ICU for a mean ± SD stay of 10.5 ± 11.05 days, in which 11 patients (5.24%) needed vasopressor support and 1 patient (0.48%) was treated with extracorporeal membrane oxygenation (ECMO). Overall, there were 26 deaths (12.4%) in our cohort (Table 4).

The Kaplan–Meier survival analysis was performed to assess the length of hospital stay and discharge rates among the study participants. This analysis revealed variations in hospital stay based on gender, type of oxygen therapy received (low nasal, high nasal, or mechanical ventilation), and vaccination status. In this case, female patients exhibited a non-significantly shorter length of hospital stay compared to male patients (p=0.872) (Figure 1).

Table 1. Baseline demographics and clinical characteristics of the included patients

Patients' demographics and baseline characteristics	*n* (%) or mean±SD (%)
Age (years)	57.43±18.4
Gender	
Male	91 (43.3)
Female	118 (56.1)
Body mass index (kg/m^2)	27.77±8.84
Smoking history	
Yes	3 (1.4)
No	50 (23.8)
Former	11 (5.2)
Unknown	144 (68.5)
Hypertension	115 (54.76)
Diabetes	94 (44.76)
Cardiovascular disease	63 (30)
Bronchial asthma	15 (7.14)
Chronic obstructive pulmonary disease	12 (5.71)
Chronic kidney disease	20 (9.52)
ESRD on dialysis	4 (1.90)
Active cancer	37 (17.62)
Human immunodeficiency virus	13 (6.19)
Solid organ transplant	53 (25.24)
Bone marrow transplant	7 (3.33)
Autoimmune disease	23 (10.95)
Liver disease	19 (9.05)
Cerebrovascular disease	20 (9.52)
Chronic neurological or neuromuscular disease	4 (1.90)
COVID vaccine	180 (85.7)
Doses given before COVID-19 diagnosis (n=178, not available=2)	
1	9 (5)
2	73 (40.6)
3	90 (50)
4	6 (3.33)
Fever	167 (80.1)
Cough	132 (62.6)
Shortness of breath	87 (41.1)
Fatigue	40 (19.1)
Muscle or body aches	16 (9)
Headache	30 (15.2)
Sore throat	41 (19.5)
Nausea or vomiting	37 (18.1)
Diarrhea	25 (11.4)
Lymphocyte count (cells/μL of blood)	3.79±21.87

(Contd...)

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

Patients' demographics and baseline characteristics	*n* (%) or mean±SD (%)
C-reactive protein (μg/mL)	106.4±326.2
Ferritin (ng/mL)	641.8±1054.3
<60	82 (39)
≥60	118 (56.2)

Table 2. Concomitant medications received with remdesivir

Concomitant medications	*n* (%)
Remdesivir+dexamethasone	101 (48.1)
Remdesivir+tocilizumab	22 (10.48)

Table 3. Oxygen therapy among the study population

Characteristics of oxygen therapy[#]	*n* (%) or Mean±SD (%)
Administration of oxygen therapy during hospitalization	96 (45.71)
Room air	129 (61)
Low-flow nasal cannula	86 (40.95)
Duration of low-flow nasal cannula (days)*	4.87±5.42
High-flow nasal cannula	22 (10.48)
Duration of high-flow nasal cannula (days)*	2.95±4.37
Mechanical ventilation	21 (10)
Duration of mechanical ventilation (days)*	15.15±16.46

Notes: [#] Indicates patient may receive more than one type of oxygen therapy; * Indicates duration of oxygen therapy=End date-Start date.

In addition, patients on mechanical ventilation were more likely to have longer periods of hospital stays (mean = 44.461 days) compared to patients with no mechanical ventilation (mean = 19.179 days) with $p<0.001$ (Figure 2).

After 30 days of hospital stays, vaccinated patients showed a higher discharge probability than non-vaccinated patients ($p=0.043$) (Figure 3).

4. Discussion

In this study, we described the demographic and clinical outcomes of hospitalized COVID-19 patients who received RDV at a hospital center in KSA. About 52.4% of our population has pneumonia. The mean ± SD age was 57.43 ± 18.4 years, and 56.1% were female. In our cohort, hypertension (54.76%), diabetes (44.76%), and cardiovascular disease (30%) were the most common comorbidities among COVID-19 patients receiving RDV. As for the clinical symptoms, the most initial common symptom of COVID-19 among our cohort was fever (80.1%), followed by cough (62.6%) and shortness of breath (41.1%). Here, 180 patients have received COVID-19 vaccine. Among them, 40.6% and 50% of patients received two and three doses, respectively.

Moreover, dexamethasone (48.1%) and tocilizumab (10.48%) were frequently administered with RDV as combination therapeutic regimens. Oxygen therapy was required in 45.7% of the patients, with 40.95% and 10.48% of patients needed low-flow and high-flow nasal

Table 4. Complications and clinical outcomes among study population

Variables	*n* (%) or mean±SD
Complications	
Bacterial pneumonia	14 (6.67)
Fungal pneumonia	2 (0.95)
Bacteremia	4 (1.9)
Acute respiratory distress syndrome	14 (6.67)
Septic shock	9 (4.29)
Clinical outcomes	
Admitted to ICU	41 (19.52)
Duration of ICU stay (days)*	10.5±11.05
Extracorporeal membrane oxygenation	1 (0.48)
Vasopressor support	11 (5.24)
Length of hospital stay (days)**	14.9±46.29
Death[#]	26 (12.4)

Notes: * Indicates duration of ICU stay=Date of ICU discharge – Date of ICU admission; ** Indicates length of hospital stay=Date of hospital discharge – Date of hospital admission; # Indicates total number of deaths=26; There is one case with no classification pneumonia or not pneumonia.
Abbreviations: SD: Standard deviation; ICU: Intensive care unit.

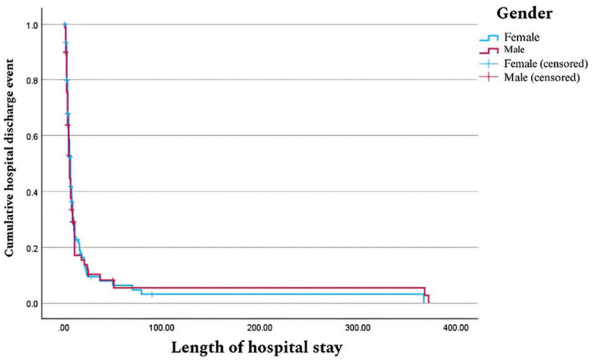

Figure 1. Kaplan–Meier curve for length of hospital stay by gender
Notes: *Censored refers to cases who did not have the event till the end of the specified or those who lost follow-up; **Censored refers to cases who did not have the event till the end of the specified time.

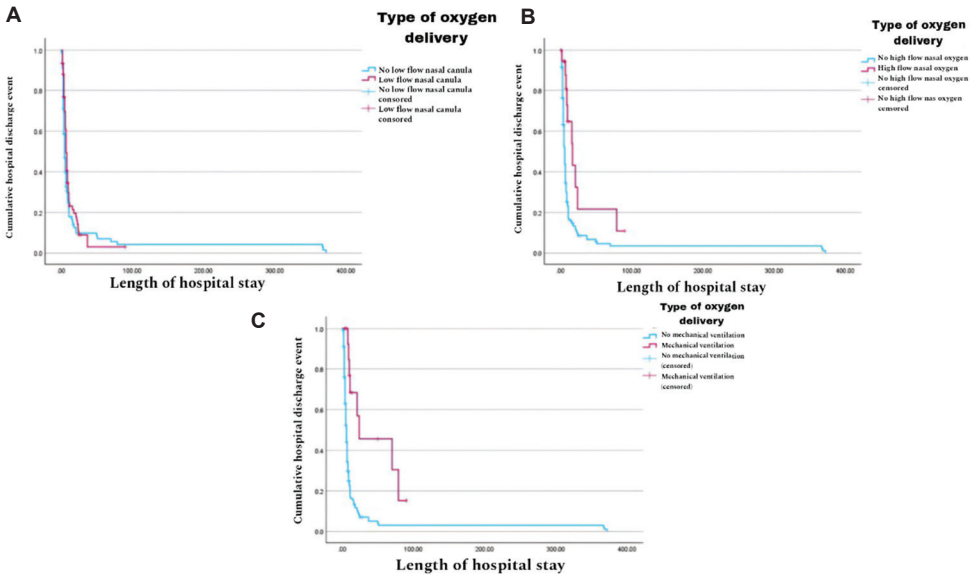

Figure 2. Kaplan–Meier estimates of hospital discharge by oxygen delivery method. (A) Low-flow nasal cannula, (B) high-flow nasal cannula, and (C) mechanical ventilation.
Notes: *Censored refers to cases who did not have the event till the end of the specified or those who lost follow-up; **Censored refers to cases who did not have the event till the end of the specified time.

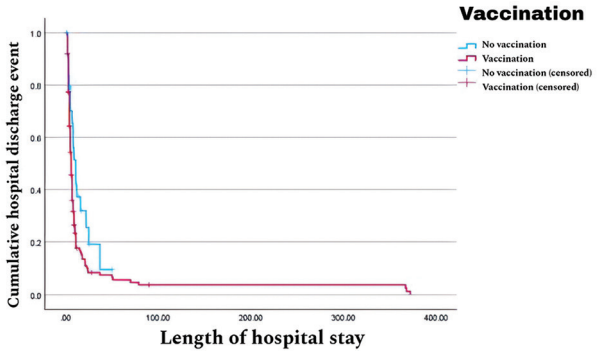

Figure 3. Kaplan–Meier curve for length of hospital stay by vaccination status
Notes: *Censored refers to cases who did not have the event till the end of the specified or those who lost follow-up; **Censored refers to cases who did not have the event till the end of the specified time.

cannulas, respectively. In addition, mechanical ventilation was required for 21 (10%) patients, 41 (19.52%) patients were admitted to the ICU, and the mean length of hospital stays among patients was 14.9 days. There were 26 (12.4%) deaths among the study participants.

In our study, the patients' age were similar to that published in other studies.[12,16,17] However, the proportion of females to males was higher than the literature.[18-20] According to Davies *et al.*, the susceptibility to COVID-19 increases with age.[21] While both males and females are equally susceptible to COVID-19 infection, males are at a higher risk for severe disease progression and poor outcomes due to the difference in inflammatory responses, hormone levels, angiotensin-converting enzyme 2 levels, and behavioral and lifestyle differences.[22]

A high proportion of patients had comorbidities, including hypertension, diabetes, and cardiovascular disease observed in our study, which is consistent with the previous studies of hospitalized patients receiving RDV.[12,23-27] This may result in a more severe form of COVID-19 due to the impact of these comorbidities on the disease progression or their high prevalence in society.[23,27] Similar to previous observations, fever was the most frequent presenting symptom among our study participants, and previous studies revealed that it is the most common symptom among hospitalized and non-hospitalized COVID-19 patients, followed by shortness of breath, dyspnea, and gastrointestinal symptoms such as nausea, vomiting, diarrhea, and emesis.[28]

The efficacy of RDV was demonstrated in different clinical trials as a combination therapy and among different populations, including hospitalized patients, outpatients, really-impaired patients, and pediatrics. Here, RDV was mainly combined with either dexamethasone or tocilizumab. The high proportion of dexamethasone and RDV administration among our cohort reflects the National Institutes of Health treatment guidelines for hospitalized COVID-19 patients. The predominant use of RDV and dexamethasone combination therapy was

reported in several studies. Ayodele *et al*. described the real-world treatment pattern in the USA from September 2020 to February 2021. They reported that dexamethasone and RDV was the most common combination therapy (14.9%) among hospitalized COVID-19 patients.[29] Similarly, Vititoe *et al*. observed that the most administered combination therapy among hospitalized COVID-19 patients (from January 2021 to February 2022) was steroids plus RDV (32.8%). However, the combination of steroids, RDV, and interleukin-6 inhibitors (such as tocilizumab) was the least administered (3.8%).[30]

The need for oxygen therapy and mechanical ventilation among our cohort was comparable to the literature. We observed that 45.7% of the patients required oxygen therapy. Among them, 40.95% and 10.48% of patients needed low-flow and high-flow nasal cannulas, respectively. Meanwhile, 10% of the participants needed mechanical ventilation, and 12.4% of the patients died. In the ACTT-1 study, 42.9%, 17.6%, and 24.2% of participants receiving RDV needed supplemental oxygen therapy, high-flow oxygen, and mechanical ventilation, respectively, and 11% of the patients died.[8] In the ACTT-2 study, supplemental and high-flow oxygen were administered to 53.3%, and 21.8% of hospitalized COVID-19 patients, respectively. Mechanical ventilation was required for 11% of the study population, and the mortality over 28 days was 7%.[31]

In contrast, Spinner *et al*. reported that only 1% of COVID-19 patients receiving 5- or 10-day RDV therapy required high-flow oxygen, and 15% and 12% of those receiving 5- and 10-day RDV therapy, respectively, needed low-flow oxygen. The authors also noted no deaths in the 5-day RDV therapy group, and 1% of the patients died in the 5-day RDV therapy group.[12] Furthermore, Ayodele *et al*. observed that only 6.3%, 2.7%, 1.4%, 1.5%, 0%, and 0.2% required ICU admission, supplemental oxygen, no-invasive mechanical ventilation, invasive mechanical ventilation, ECMO, and vasopressor use, respectively, which is lower than our own observation.[29]

The mean length of hospital stay in our study (14.9 days) was comparable to that in the literature, as it was 10, 13, 15, and 17 days in Ali *et al*., Karolyi *et al*., Tejada *et al*., and Beigel *et al*., respectively.[8,13,32,33] The findings regarding the impact of RDV on the length of hospitalization were contradictory. Several studies have highlighted that the length of hospital stay was significantly longer among patients receiving RDV.[32,34]

The strength of our study is that it offers an overall picture of the management and outcomes of hospitalized COVID-19 patients treated with RDV over a long period of time (from January to December 2021), including the period of approval for RDV in KSA. In addition, the study participants were real-life cohort of hospitalized patients due to COVID-19 with high prevalence of comorbidities. However, our study has limitations, including the retrospective single-center study design and small sample size. There was lack of control group to examine the clinical utility of RDV compared to the standard care or other treatment regimens. Moreover, association studies between RDV use, demographic, and clinical characteristics of the patients was not conducted, which may affect the interpretation of our findings. All these factors would introduce bias, cause unavoidable residual confounding effects, and limit the generalizability of our findings.

5. Conclusion

As with most studies, our cohort showed that the possibility of COVID-19 infection increases with age. Although people with different comorbidities are more susceptible to COVID-19 infection, people without comorbidities are also in danger. Moreover, the dangers and severity of the disease are not only present due to its different symptoms but also to its ability to make the patients more vulnerable to different lung infections, which easily lead to pneumonia. RDV is a promising antiviral therapy against COVID-19 infection and, in most cases, dexamethasone and tocilizumab were combined with RDV. The overall mortality and ICU admission rates were lower than previously reported. In addition, the overall length of hospital stays was comparable to the literature.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

This study was reviewed and approved by the Research Ethics Committee at King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia (Approval ID: IRB 2023 – 33). Given the retrospective nature of the study and use of hospital medical records, the requirement for written informed consent was waived by the REC. All patient data were handled confidentially in compliance with institutional and national ethical standards.

Consent for publication

As this study involved retrospective analysis of de-identified patient data without any identifiable images, individual informed consent for publication was not required and waived by Research Ethics Committee at King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia.

Availability of data

Clinical researchers may request to gain access to the patient-level data and related study documents. Data used in this work are available from the corresponding author on reasonable request. Before sharing, patient-level data will be anonymized, and study documents will be redacted to protect the privacy of trial participants.

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ACCSCIENCE PUBLISHING

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ORIGINAL ARTICLE

Household arsenic and acrolein exposures and risk of urothelial cell carcinoma

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Abstract

Background: Smoking accounts for about half of bladder cancer cases in the United States; however, the etiology of up to one-third of urothelial cell carcinoma (UCC) remains poorly understood. Acrolein and arsenic are known bladder carcinogens with documented household exposures. **Aim:** This study aimed to (i) determine whether urinary and household exposures to acrolein and inorganic arsenic (iAs) are higher in newly diagnosed UCC patients than in those with benign urologic disease, (ii) assess whether urinary concentrations reach genotoxic thresholds, and (iii) evaluate how these exposures vary by urbanicity and area deprivation indices. **Methods:** Patients were recruited from the Urology Clinic at the University of Wisconsin–Madison and provided urine, drinking water, and household dust samples. **Results:** Acrolein exposures (as its stable metabolite 3-hydroxy-propyl-mercapturic acid) did not differ between cases and controls. Urinary arsenic concentrations were higher in cases than in controls but did not reach statistical significance (p=0.08). Unadjusted urinary iAs concentrations (reflecting urothelial exposures) ranged from 0.01 to 0.71 μM in cases and 0.02 – 0.14 μM in controls (p=0.05). No patients reached genotoxic urinary concentrations of iAs (10 μM) at a single time point. Arsenic concentrations in household dust were higher in UCC (0.42 ng/cm^2) compared to control households (0.29 ng/cm^2; p=0.007). Dust arsenic levels also correlated with urinary iAs across all patients (r = 0.41; p=0.004). Drinking water arsenic was associated with higher area deprivation percentiles (r = 0.30, p=0.046) and with households from more rural areas (p=0.039) but did not differ significantly between cases and controls. **Conclusion:** Our data suggest that indoor dust arsenic, rather than arsenic in drinking water, was a likely source of urinary arsenic exposure in this primarily non-smoking population. **Relevance for patients:** Simple in-home arsenic mitigation strategies, such as using high-efficiency particulate air vacuum cleaners and air filtration units, may help reduce exposure for patients diagnosed with UCC.

Keywords: Bladder cancer; Non-smokers; Household dust; Inorganic arsenic; Drinking water

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1. Introduction

Urothelial cell carcinomas (UCCs) represent the majority of human bladder tumors[1] and are considered environmental cancers.[2] Cigarette smoking accounts for approximately 50% of bladder cancer cases in both men and women,[3] with 2 – 8% of cases attributed to occupational exposures.[1,4,5] However, about one-third of bladder cancer cases in the United States remain unexplained by these known risk factors.

The incidence of UCC is higher among individuals from lower socioeconomic strata,[6-8] even independent of smoking status,[9,10] though the underlying reasons for this disparity are not fully understood. UCC risk is also elevated in populations living in areas of higher industrial activity,[1,11] which may lead to increased environmental exposure to pollutants in soil, water, or air, including known bladder carcinogens such as arsenic and acrolein.[2,12] Inorganic arsenic (iAs) is found in air pollution, second-hand tobacco smoke, contaminated drinking water, certain foods, and even household dust.[13-15] Acrolein is present in automobile exhaust, industrial emissions, second-hand smoke, wood fires, cooking fumes, and fried foods.[16] However, urinary arsenic and acrolein exposures have not been evaluated in individual bladder cancer patients in the United States.

We recently found that all 42 primarily non-smoking healthy adults in a previous study had measurable urinary exposures to iAs and acrolein, with some reaching genotoxic concentrations as assessed *in vitro* using primary and immortalized urothelial cells.[17,18] Furthermore, these urinary exposures correlated with those in their pet dogs, suggesting household, rather than occupational, sources for these environmental chemicals.[17]

We hypothesized that household exposures to arsenic and acrolein contribute to non-tobacco, non-occupational UCC risk. The aims of this study were to determine whether urinary and household exposures to acrolein and iAs are higher in patients with newly diagnosed UCC compared to those with benign urologic disease, to assess whether urinary concentrations reach genotoxic urothelial thresholds, and to characterize urinary and household chemical exposures by urbanicity and area deprivation indices.

2. Methods

2.1. Patient recruitment

Patients with a biopsy-confirmed diagnosis of superficial or muscle-invasive UCC were enrolled from the urology clinic at the teaching hospital of the University of Wisconsin–Madison (UWHealth). Controls were patients from the same clinic with benign urologic disease (kidney stones, benign prostatic hyperplasia, urinary incontinence) and were recruited to be comparable in age, sex, and race to UCC patients. All enrolled patients were self-reported current non-smokers (never smokers or former smokers who had quit at least 12 months prior).[19]

All study procedures were approved by the University of Wisconsin–Madison Institutional Review Board (protocol number 2022-1030-CP002), and all patients provided written informed consent. Voided urine samples (minimum 15 mL) were collected from each patient, either as leftover specimens from routine urinalysis or through home collection. Patients were also provided a kit containing a questionnaire and materials to collect drinking water and household dust samples. For water sampling, patients were asked to collect a sample from their primary drinking water source using a provided 4-ounce plastic bottle. Tap water was run for 2 min before sample collection; bottled or fridge-filtered water was collected directly. For dust sampling, patients received disposable gloves, a 25 cm × 25 cm paper template, and a Ghost Wipe™ (SKC Inc.), with instructions to collect dust from the threshold of the primary bedroom after refraining from vacuuming for 1 week. Dust wipes were placed in 50 mL conical tubes. All samples were returned to the principal investigator's laboratory through overnight mail in a postage-paid box.

2.2. Chemical assays

Chemical analyses were performed at the Wisconsin State Laboratory of Hygiene at the University of Wisconsin–Madison. Urinary acrolein exposure was quantitated by measuring its stable 3-hydroxy-propyl-mercapturic acid (3-HPMA) metabolite, using liquid chromatography-electrospray ionization-mass spectrometry.[20] Total arsenic was measured in dust and drinking water, while iAs species – including arsenite, arsenate, monomethylarsonic acid, dimethylarsinic acid, and trimethylarsine oxide – were measured in urine using liquid chromatography-inductively coupled plasma-mass spectrometry.[14] Urinary cotinine was included as a marker of active smoking or second-hand tobacco smoke exposure.[21] Concentrations of all urinary chemicals were normalized to urinary creatinine (creat) to control for individual differences in urine concentration.[17] Arsenic concentrations in household dust were normalized to the sampled surface area.

2.3. Questionnaires

Patients were asked to complete a questionnaire addressing demographic information (age range by decade, sex, race, and current/previous occupations), as well as household factors relevant to acrolein and arsenic exposures.

Questions covered tobacco smoking, type of neighborhood (urban/suburban/rural), degree of drive-by automobile traffic, frequency of home herbicide and insecticide use, primary drinking water sources, and the presence of a treated lumber deck, swimming pool, or wood-burning fireplace. Each questionnaire was assigned a unique household study ID to protect patient confidentiality.

2.4. Area deprivation and urbanicity

We used the area deprivation index (ADI) as an indicator of socioeconomic status for each patient. The ADI incorporates aggregate income, education, employment, and housing quality by census block.[22] The online Neighborhood Atlas® tool[23] was used to map home addresses to ADI scores by national percentiles. A higher ADI and larger national percentiles indicate greater poverty and deprivation based on income, education, employment, and housing quality. Compiled data from the Neighborhood Atlas were available from 2017 to 2021. To assess urbanicity, we assigned the United States Department of Agriculture Rural-Urban Continuum Codes (RUCC) based on each patient's home address. For this system, codes 1 – 3 correspond to metropolitan (urban) areas and codes 4 – 9 correspond to increasingly rural areas.[24] The United States Department of Agriculture RUCC data were available for 2023.[25]

2.5. Statistical analyses

Questionnaire data were encoded as categorical outcomes and compared between cases and controls using Chi-square or Fisher's exact tests, with odds ratios (OR) and 95% confidence intervals (CI). Urinary 3-HPMA and iAs species, arsenic concentrations in dust and drinking water, and national percentiles for the ADI and RUCC were compared between UCC cases and benign urologic controls using Mann–Whitney tests. Urinary and household chemical concentrations were correlated across all patients by ADI and RUCC using Spearman correlation tests.

Total urinary iAs and 3-HPMA concentrations for each patient were compared to *in vitro* genotoxic thresholds in primary human urothelial cells: 10 μM for iAs and 1.1 μM for acrolein.[18] Percentages of cases and controls reaching genotoxic urinary chemical concentrations were compared using Fisher's exact tests.

3. Results

3.1. Patient demographics

Patients with UCC and control patients with benign urologic disease had comparable racial and ethnic backgrounds (primarily Caucasian, non-Hispanic) and annual income levels (Table 1). However, patients with UCC were less likely to have completed any post-college education compared to patients with benign urologic disease (p=0.0013; Table 1).

Table 1. Demographic characteristics of patients with urothelial cell carcinoma and control patients with benign urologic disease

Characteristic	UCC patients *n*=25	Benign controls *n*=25
Age range		
41 – 50	1	2
51 – 60	0	2
61 – 70	10	6
71 – 80	13	14
81+	1	1
Sex		
Male	19	20
Female	6	5
Race		
Caucasian	23	24
No response	2	1
Ethnicity		
Non-Hispanic	23	20
Hispanic	0	1
No response	2	4
Highest educational level		
Post-college	2	13*
Four-year college	8	2
Some college	7	6
High school	6	3
No response	2	1
Annual income[a]		
<$52,000	4	1
$52,000 – 156,000	16	16
>$156,000	1	6
No response	4	2
Median years lived at current address (range)	22 years (1 – 49 years)	20 years (5 – 48 years)

Notes: [a]Annual income in USD; *indicates statistical significance at p=0.0013.
Abbreviation: UCC: Urothelial cell carcinoma.

3.2. Questionnaire data

Patient responses to the household questionnaires are listed in Table 2. There were no significant differences in the history of industrial employment, reported drive-by traffic near the home, use of an indoor fireplace, wood-burning stove, or swimming pool, use of a well for drinking water,

Table 2. Questionnaire data from 25 patients with urothelial carcinoma and 25 controls with benign urologic disease

Category	Responses	UCC, *n* (%)	Benign, *n* (%)	Odds ratio (95% confidence interval)	*p*-value
Industrial occupation	Respondents	22	24	0.85 (0.29 – 2.92)	>0.99
	Yes	11 (50.0)	13 (54.2)		
	No	11 (50.0)	11 (45.8)		
Applied pesticides to crops	Respondents	22	24	0.2 (0.4 – 1.12)	0.07
	Yes	2 (9.1)	8 (33.3)		
	No	20 (90.9)	16 (66.6)		
Weedkiller use at home	Respondents	23	24	0.17 (0.05 – 0.66)	0.008*
	Yes	9 (39.1)	19 (79.2)		
	No	14 (60.9)	5 (20.8)		
Drive-by traffic near home	Respondents	22	24	0.50 (0.16 – 1.72)	0.37
	Moderate/heavy	10 (45.5)	15 (62.5)		
	Minimal	12 (55.5)	9 (37.5)		
Treated lumber porch or deck	Respondents	22	24	1.02 (0.33 – 3.03)	>0.99
	Yes	12 (55.5)	13 (54.2)		
	No	10 (45.5)	11 (45.8)		
Swims in a pool	Respondents	22	23	1.05 (0.33 – 3.03)	>0.99
	Yes	2 (9.1)	2 (8.7)		
	No	20 (90.9)	21 (91.3)		
Wood-burning fireplace	Respondents	22	24	0.47 (0.14 – 1.65)	0.24
	Yes	7 (31.8)	12 (50.0)		
	No	15 (68.2)	12 (50.0)		
Tobacco smoking	Respondents	22	24	1.68 (0.49 – 5.04)	0.56
	Former	12 (54.5)	10 (41.7)		
	Never	10 (45.5)	14 (58.3)		
Well water for drinking (any)	Respondents	25	24	0.61 (0.17 – 2.36)	0.52
	Yes	5 (20.0)	7 (29.2)		
	No	20 (80.0)	17 (70.8)		
Water filtration	Respondents	22	24	1.68 (0.49 – 5.04)	0.56
	No	12 (54.5)	10 (41.7)		
	Yes	10 (45.5)	14 (58.3)		

Note: *Indicates statistical significance ($p<0.05$).
Abbreviation: UCC: Urothelial cell carcinoma.

or smoking status (former versus never). Only one subject from each group had worked as a hairdresser or owned a swimming pool (both controls). Observably, fewer UCC cases reported a history of applying pesticides to crops (9.1%) compared to control patients (33.3%), but this difference did not reach significance ($p=0.074$; Table 2). Contrary to our hypotheses, UCC patients reported less herbicide use at home than control patients (OR: 0.17; 95% CI: 0.05 – 0.66).

3.3. Acrolein metabolites in urine

Urinary concentrations of the acrolein metabolite 3-HPMA did not differ between patients with UCC (median: 193 nmol/mg creat, range: 65 – 899 nmoL/mg creat) and those with benign urologic disease (median: 195 nmoL/mg creat, range: 53 – 1,059 nmoL/mg creat) ($p=0.44$; Figure 1).

When unadjusted urinary 3-HPMA concentrations were assessed for possible genotoxicity, 9 of 25 UCC

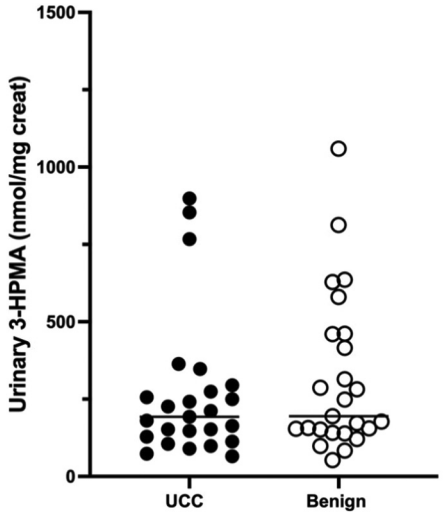

Figure 1. Urinary concentrations of the acrolein metabolite 3-hydroxypropyl-mercapturic acid in patients with urothelial cell carcinoma and those with benign urologic diseases. Horizontal lines indicate median values. p=0.44 between groups.

patients (36%; 3-HPMA concentrations of 1.17 – 7.20 μM) and 12 of 25 controls (48%; 3-HPMA concentrations of 1.23 – 4.79 μM; p=0.57) reached the genotoxic threshold of 1.1 μM[18] for possible urinary acrolein exposure. Median urinary 3-HPMA concentrations were not higher in households with fireplaces or wood-burning stoves (p=0.56) or in areas with reported moderate-to-heavy traffic (p=0.64; data not shown). In addition, urinary 3-HPMA was not higher in patients living in more urban neighborhoods (RUCC 1 – 3; 179 nmoL/mg creat) compared to those in less urban neighborhoods (RUCC 4 – 9; 262 nmoL/mg creat; p=0.55).

3.4. iAs in urine, dust, and water

Total iAs species in urine (the molar sum of arsenite, arsenate, dimethylarsinic acid, monomethylarsonic acid, and trimethylarsine oxide) did not differ significantly between UCC cases (0.070 nmoL/mg creat; range: 0.020 – 0.680) and controls (0.060 nmoL/mg creat, range: 0.018 – 0.419; p=0.08), although some individual UCC patients had outlier high concentrations of urinary iAs (Figure 2A). Unadjusted urinary iAs concentrations (reflecting urothelial exposures) ranged from 0.01 to 0.71 μM in cases and 0.02 – 0.14 μM in controls (p=0.05; Figure 2B). No subjects had unadjusted urinary iAs concentrations that reached the genotoxic threshold of 10 μM.[18]

Arsenic levels in indoor dust were significantly higher in the homes of patients with UCC (0.424 ng/cm^2; range: 0.165 – 2.30) compared to controls (0.292 ng/cm^2; range: 0.146 – 0.730) (p=0.007; Figure 3A). Furthermore, household dust arsenic was positively correlated with urinary iAs across all subjects (r = 0.41; p=0.004; Figure 3B). There was no significant difference in household dust arsenic between households reporting moderate-to-heavy traffic versus minimal traffic (p=0.86, data not shown).

Concentrations of arsenic in drinking water ranged from <0.04 to 5.05 μg/L, with no significant difference between case (median: 0.135 μg/L) and control households (median: 0.123 μg/L; p=0.61; Figure 4). Arsenic in drinking water did not correlate with iAs across all subjects (r = −0.24, p=0.28).

3.5. Urine cotinine

Three UCC patients and two controls had detectable cotinine levels in their urine, ranging from 2 to 5,473 ng/mg creatinine. Of these five individuals, four were former smokers, but only one reported recent smoke exposure on the questionnaire. There were no consistent observable differences in urinary 3-HPMA, urinary arsenic, or dust arsenic between patients who tested positive for urinary cotinine and those who did not (data not shown).

3.6. ADI and urbanicity data

Patients with UCC tended to reside in lower-resourced neighborhoods (median area deprivation percentile: 38.0%, range: 11.0 – 94.0%) compared to controls (median area deprivation percentile 31.5%, range: 10.0 – 69.0%), although this difference was not statistically significant (p=0.11; Figure 5). Area deprivation percentiles showed a modest correlation with higher drinking water arsenic concentrations (r = 0.30, p=0.046; Figure 6A) but were not associated with dust arsenic (p=0.51), urinary iAs (p=0.42), or urinary 3-HPMA (p=0.52).

The median RUCC (urbanicity score) was 2 for both cases (range: 2 – 9) and controls (range: 1 – 4), with no significant difference between groups (p=0.24). RUCC was not significantly correlated with arsenic levels in drinking water (r = 0.24, p=0.11), indoor dust (r = 0.05, p=0.74), urinary iAs (r = −0.16, p=0.29), or urinary 3-HPMA (r = 0.14, p=0.35). In sub-analyses, higher urbanicity scores (RUCC 1 – 3) were not associated with higher urinary iAs (p=0.94), dust arsenic (p=0.97), or urinary 3-HPMA (p=0.55; data not shown). However, arsenic levels in drinking water were significantly higher in households located in more rural areas (RUCC 4 – 9; 0.42 μg/L) compared to urban areas (RUCC 1 – 3; 0.12 μg/L; p=0.046; Figure 6B).

4. Discussion

Acrolein and iAs are both established bladder carcinogens[26,27] and can be detected in the urine of healthy

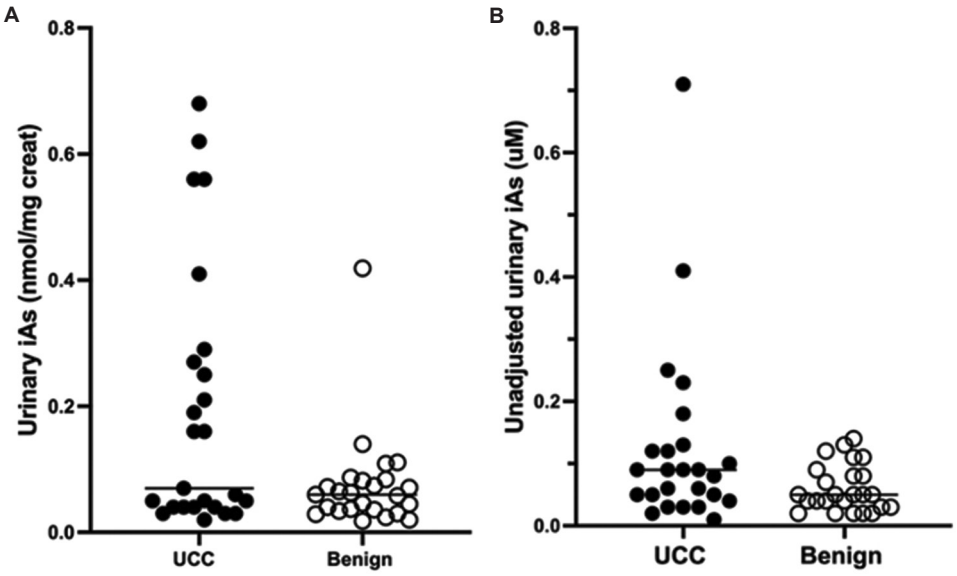

Figure 2. Inorganic arsenic (iAs) exposures in patients with urothelial cell carcinoma (UCC) and those with benign urologic diseases. Horizontal lines indicate median values. (A) Urinary total molar iAs species, normalized to urine creatinine (p=0.08 between groups). (B) Unadjusted urinary inorganic arsenic concentrations, reflecting urothelial exposures, in UCC cases (median 0.09 μM) and benign controls (0.05 μM; p=0.05).

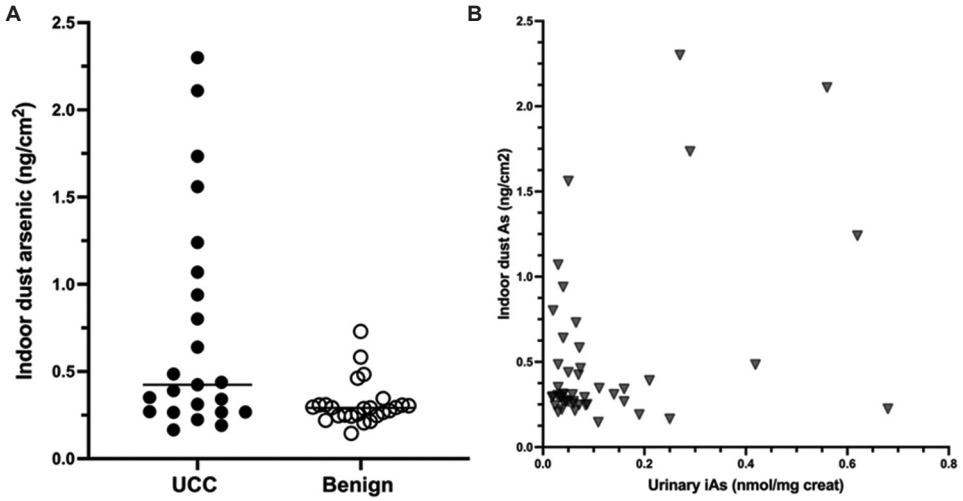

Figure 3. Arsenic (As) concentrations in household indoor dust for patients with urothelial cell carcinoma and those with benign urologic diseases. (A) Indoor dust As concentrations between groups (p=0.007). (B) Correlation between indoor dust As and urinary inorganic arsenic (iAs) concentrations across all subjects (r = 0.41; p=0.004).

subjects under non-tobacco, non-occupational household exposure conditions.[17] In the current study, iAs and the acrolein metabolite 3-HPMA were detected in the urine of all 50 urologic patients evaluated.

Urinary iAs species, measured at a single time point, did not differ significantly between UCC cases and controls; however, some UCC patients exhibited outlier-high urinary concentrations of iAs. Due to this variability, a *post hoc* sample size calculation (www.stat.ubc.ca) estimated that 47 UCC patients and 47 controls would be needed to show statistical significance for the observed difference in urinary iAs concentrations. Other studies have reported higher total arsenic (organic and inorganic) and a greater percentage of iAs in the urine of UCC cases versus controls, though more than half of the enrolled cases in those studies were smokers.[28]

We previously demonstrated that iAs is genotoxic to human urothelial cells at concentrations ≥10 μM.[18] In this study, unadjusted urinary iAs concentrations, which reflect urothelial exposures, reached as high as 0.71 μM in cases,

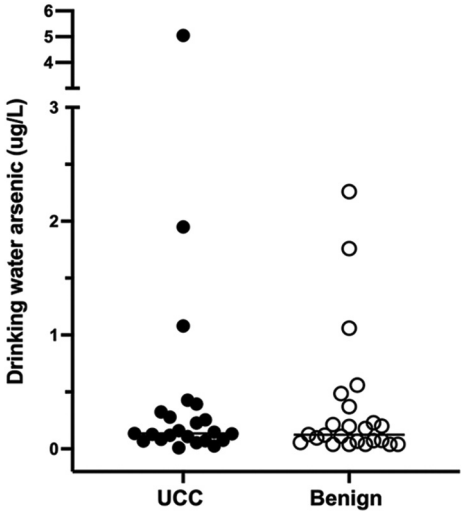

Figure 4. Directly measured arsenic concentrations in household drinking water among patients with urothelial cell carcinoma and those with benign urologic disease (p=0.58 between groups)

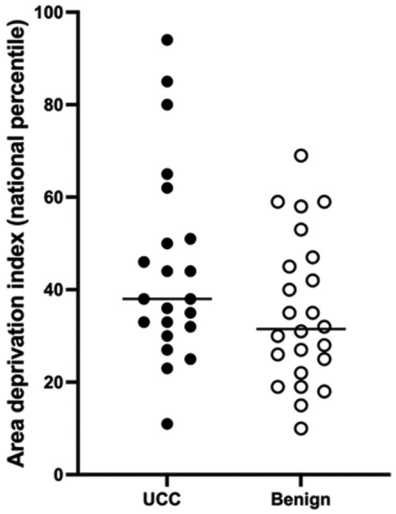

Figure 5. Area deprivation index, based on home address, as a measure of lower socioeconomic resources for patients with urothelial cell carcinoma and those with benign urologic diseases. The Y-axis represents area deprivation by national percentile (higher percentiles correspond to lower socioeconomic resources). p=0.11 between groups.

with none approaching the 10 μM genotoxic threshold, at least at a single sampling time point. Other studies have not differentiated arsenic species concentrations by molarity, making it difficult to assess whether similar genotoxic levels were reached in any other patient populations.[28,29] Our genotoxic threshold was established *in vitro* using a 6-h exposure in a primary human urothelial cell line, with DNA damage assessed through the comet assay.[18] A prior study in immortalized SV40 human urothelial cells incubated with iAs for 48 h identified a lower genotoxic threshold of 1 μM using the same assay,[30] though this level was not observed in our patient cohort. However, iAs also exert non-genotoxic effects such as oxidative stress and inhibition of DNA repair,[31,32] and the thresholds for these effects remain poorly defined in primary urothelial cells.

Arsenic was detected in all indoor dust samples, with significantly higher concentrations observed in UCC households compared to controls. In addition, dust arsenic levels were positively correlated with urinary arsenic species across all patients. This suggests that indoor dust may be a relevant source of urinary arsenic exposure in this primarily non-smoking population. Arsenic accumulates in indoor dust through airborne particulate matter originating from sources of outdoor soil and roadway dust[33] and may be absorbed through inhalation or unintentional ingestion.[34] Although our study did not find an association between indoor dust arsenic and RUCC, a study from China reported higher indoor dust arsenic levels in urban versus rural areas.[35] Practical measures to reduce indoor dust arsenic exposure include removing shoes at the home entrance, using high-efficiency particulate air (HEPA)-filtered vacuum cleaners, and installing HEPA air filtration units.[36,37]

Arsenic was also detectable in nearly all drinking water samples, with higher levels found in homes located in more rural areas and those with higher area deprivation scores. These findings are consistent with previous studies reporting higher arsenic concentrations in community water systems serving small, rural populations,[38] and in regions with greater socioeconomic vulnerability.[39] However, arsenic levels in drinking water did not differ between UCC and control households, nor did they correlate with urinary arsenic concentrations at a single time point. Importantly, none of the water samples exceeded the Environmental Protection Agency's regulatory limit for arsenic (10 μg/L).[40] High groundwater arsenic levels have been linked to clusters of human bladder cancer in Chile,[41] where average water concentrations ranged from 43 to 94 μg/L. However, urinary arsenic concentrations in those populations were not reported. These previously reported water arsenic concentrations were more than four times higher than the highest value observed in our study (5.1 ug/L) at a single time point. Future studies would benefit from longitudinal sampling of drinking water to better assess chronic exposure.

The stable urinary acrolein metabolite 3-HPMA did not differ between UCC cases and benign urologic controls in the current study. This finding was contrary to our hypothesis, as acrolein-DNA adducts have been documented in human bladder tumors.[12,42] Only one other study has compared

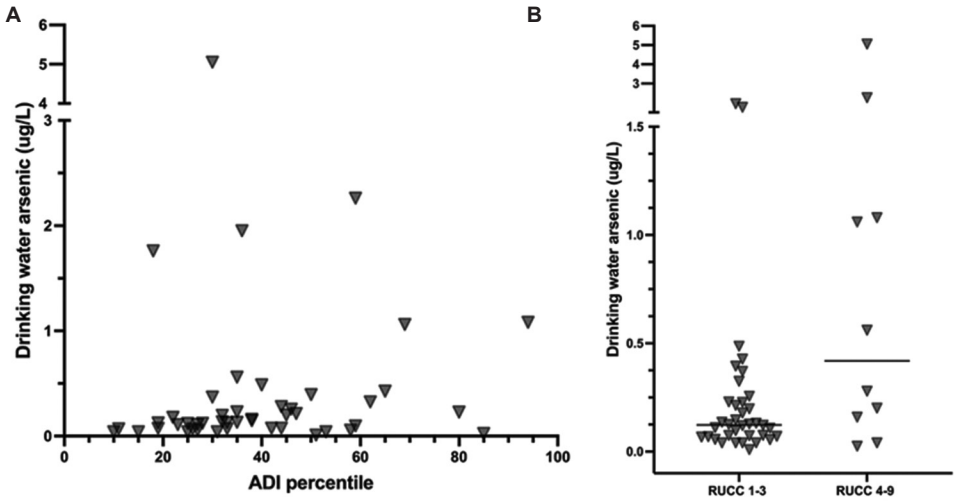

Figure 6. Drinking water arsenic concentrations across 50 patients with urothelial cell carcinoma or benign urologic diseases, categorized by (A) area deprivation index (ADI; p=0.11) and (B) the United States Department of Agriculture Rural-Urban Continuum Codes (RUCC; 1 = Most urban; p=0.046 between groups)

urinary 3-HPMA levels between UCC and control patients. While their concentrations were comparable to the medians observed in our population (193 – 195 ng/mg creat), the authors paradoxically found lower urinary 3-HPMA/creat concentrations in UCC cases versus controls.[42] However, the analyzed UCC patients had chronic kidney disease, which could have affected urinary 3-HPMA excretion.

We did not observe differences in urinary 3-HPMA between patients living in urban versus rural areas. Although acrolein is generated from vehicular exhaust, it is also a component of indoor air pollution, especially in smoking households.[43] Although all patients in our study were self-reported current non-smokers, urinary cotinine was detected in 5 of 50 subjects. Therefore, we cannot rule out tobacco smoke or electronic cigarettes[44] as sources of urinary 3-HPMA in some participants. Other major sources of acrolein in non-smoking households include heated cooking oils and wood-burning fireplaces.[45]

Patients with UCC in our study were less likely to have completed any post-college education compared to controls from the same urology clinic. This finding is consistent with larger studies reporting lower educational attainment among UCC patients compared to unaffected controls,[10,46] even when adjusted for smoking.[9] The reasons for higher UCC risk among individuals with lower educational attainment are not fully understood.

Our study has several limitations, including a small sample size and the collection of samples at a single time point. Some patients had lived in their current homes for a short duration, and we did not have access to household samples from previous residences. Follow-up studies with a larger sample size are warranted, ideally with the assessment of urinary chemical exposures at multiple time points. It would also be beneficial to collect more detailed information on potentially confounding UCC risk factors, such as diet or total trihalomethane exposure from municipal water,[47] and to include additional urinary chemical exposures, such as aromatic amines.[48]

In addition, while none of the patients had genotoxic urinary concentrations of iAs, our genotoxic threshold was based on DNA strand breaks.[18] Arsenic exposure thresholds for oxidative stress and inhibition of DNA repair still need to be established in human urothelial cells.

Overall, we found that patients with UCC had higher concentrations of arsenic in indoor household dust, which significantly correlated with urinary iAs concentrations across all subjects. Although drinking water arsenic levels were higher in households located in more rural areas and with higher area deprivation scores, these were not associated with urinary arsenic concentrations or UCC in our study population. Our data suggest that indoor dust arsenic, rather than arsenic in drinking water, was a likely source of urinary arsenic exposures in this primarily non-smoking population. Simple home arsenic remediation strategies, such as using HEPA vacuum cleaners and HEPA air filtration units, should be considered for patients diagnosed with UCC.[36,37]

5. Conclusion

Arsenic in household dust is associated with urinary iAs concentrations and may contribute to UCC risk in non-smokers. The impact of household arsenic remediation

on urinary arsenic concentrations warrants further investigation.

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Conflict of interest

The authors declare no conflicts of interest.

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Writing – original draft: Lauren Trepanier, Hannah Peterson
Writing – review & editing: All authors

Ethics approval and consent to participate

All study procedures were approved by the University of Wisconsin–Madison Institutional Review Board (protocol number 2022-1030-CP002), and all patients provided written informed consent.

Consent for publication

The research subjects gave consent to publish their de-identified data.

Availability of data

Data used in this work are available from the corresponding author upon reasonable request.

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