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Exploring The Contribution Of The Skeletal Stem Cell To Spinal Fusion: From Clinical Practice to Pathophysiology.

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A thesis submitted to the University of Dublin in partial fulfilment
of the requirements for the degree of

Doctor of Philosophy, Ph.D.

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Declaration

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Summary

As global demographics shift towards an ever increasingly aged population, the prevalence of age-related orthopaedic and spinal conditions has increased substantially. Conditions, such as osteoporosis and frailty, have an increased incidence within the ageing population. These conditions not only elevate the risk of fracture and deformity but also increase the complication rates during surgical interventions which involve bony fusion. Spinal fusion, aimed at stabilizing the spine by joining two or more vertebrae, is a well-established technique, but its success is heavily influenced by the patient's bone quality and overall health status. With the rising demand for spinal surgeries among older patients, there is a critical need for innovations that improve the outcomes of these procedures.

This thesis, *“Exploring The Contribution Of The Skeletal Stem Cell To Spinal Fusion: From Clinical Practice to Pathophysiology,”* addresses these challenges by exploring both clinical advancements and biological techniques that can enhance spinal fusion outcomes, particularly in vulnerable populations. One of the key areas of investigation is the potential role of human skeletal stem cells (hSSCs) in improving bone healing and promoting successful fusion, offering a promising alternative to traditional bone grafts and mesenchymal stromal cells (MSCs).

The research spans from clinical practice to laboratory investigations, providing a holistic view of the current landscape of spinal fusion, with particular emphasis to spinal surgery in Ireland. It also introduces novel diagnostic tools to assess bone health and explores the latest technological advancements in surgical techniques, such as robotic-assisted surgery and image-guided navigation. By investigating both the biological and technological aspects of spinal fusion, this thesis aims to bridge gaps between clinical needs and emerging therapies, with a particular focus on the therapeutic potential of hSSCs in regenerative medicine.

The thesis begins with a detailed review of the anatomy of the spine and tissues involved in spinal fusion, including bone, intervertebral discs, ligaments, and the spinal cord. It explains the processes of bone formation, remodelling, and repair mechanisms, focusing on skeletal stem cells' potential applications in tissue engineering and regenerative medicine. Following this, a historical review of spinal fusion is provided, from early treatments to modern-day advancements. It covers the evolution of techniques such as pedicle screw fixation and robotic-assisted surgeries. The chapter also discusses

the complications associated with spinal fusion, including pseudarthrosis and the importance of precision in surgical techniques. Chapter 4 reviews modern technologies, such as image-guided navigation and robotic-assisted surgery, highlighting their role in improving precision and outcomes in spinal fusion surgeries. It also explores cost-effectiveness and future developments, including augmented reality in surgical navigation.

Chapters 5 and 6 address the increased risks in performing spinal fusion on osteoporotic and frail patients, exploring the epidemiology, diagnosis, and interventions for osteoporosis, sarcopenia, and frailty. Emphasis is placed on optimizing surgical preparation for these vulnerable groups.

Chapters 7 and 8 investigate the current evidence on potential alternatives to iliac crest bone autograft. Chapter 7 compares iliac crest bone grafts to cell-based grafts, while Chapter 8 compares recombinant human bone morphogenetic proteins (rhBMP) to traditional bone grafts. These sections analyse the outcomes of these techniques in spinal fusion and evaluate their effectiveness in promoting bone healing and fusion.

Chapter 9 includes a meta-analysis on the diagnostic accuracy of Hounsfield units (HU) in computed tomography compared to dual-energy X-ray absorptiometry (DEXA) for diagnosing osteoporosis in spinal surgery patients, emphasizing the clinical utility of Hounsfield units for preoperative assessment and identifying these higher risk patients. Chapter 10 further investigates the use of the HU as a means of predicting those patients at risk of mal- or non-union for specific cases of anterior cervical discectomy and fusion.

The optimal method of harvesting cells for fusion procedures remains elusive. In Chapter 12 we propose a new technique, navigation-assisted vertebral body bone marrow aspiration, which allows for intraoperative harvesting for immediate use of cells intraoperatively, supported by an analysis of mononuclear cell yields and osteogenic differentiation potential.

A core focus of the thesis is the exploration of the human skeletal stem cell (hSSC) as a potential superior alternative to mesenchymal stromal cells (MSCs) in bone tissue engineering and this is explored in Chapter 13. We discuss the identification and characterization of hSSCs, their role in skeletal repair, and their potential applications in improving spinal fusion outcomes.

The thesis aims to bridge clinical practice with pathophysiology, emphasizing the role of skeletal stem cells in advancing spinal fusion techniques. By addressing current

diagnostic challenges and introducing innovative surgical approaches, the thesis offers excitement for the future adoption of SSC-based therapies to improve patient outcomes, particularly in the context of an aging population.

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Publications & Abstracts

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Nomenclature

AF	Annulus Fibrosis
ALL	Anterior Longitudinal Ligament
BIA	Bioelectric Impedance Analysis
BM	Bone Marrow
BMA	Bone Marrow Aspirate
BMD	Bone Mineral Density
BMP-2	Bone Morphogenic Protein-2
CBT	Cell Based Therapies
CEP	Cartilage End Plates
CGA	Comprehensive Geriatric Assessment
CT	Computed Tomography
DEXA	Dual-Energy X-ray Absorptiometry
ECM	Extracellular Matrix
EFS	Edmonton Frailty Scale
FI	Frailty Index
mFI	Modified Frailty Index
FP	Frailty Phenotype
FFI	Fried Frailty Index
GH	Growth Hormone
HRQoL	Health-Related Quality of Life
HU	Hounsfield Unit
ICBG	Iliac Crest Bone Graft
IGF-1	Insulin-like Growth Factor 1
IL-6	Interleukin-6
ISCT	International Society For Cellular Therapy
IVD	Intervertebral Disc
MRI	Magnetic Resonance Imaging
MSC	Mesenchymal Stromal Cells
NP	Nucleus Pulposus
OP	Osteoporosis
PLL	Posterior Longitudinal Ligament
POPS	Proactive Care for Older Persons Undergoing Surgery
QCT	Quantitative Computed Tomography
QOL	Quality of Life
rhBMP	Recombinant Bone Morphogenetic Proteins
SPPB	Short Physical Performance Battery
SSC	Skeletal Stem Cell
TNF-A	Tumour Necrosis Factor Alpha
TUG	Timed-Up-and-Go-Test

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Chapter 1 Introduction

1.1 Our Aging Population

The increasing surge in the aging population presents an unprecedented challenge in global health dynamics^{1,2}. As per recent demographic trends, societies are experiencing a significant shift with a growing proportion of individuals aged 65 and older, a phenomenon more pronounced in developed nations but rapidly escalating too in developing countries³. This demographic change, profoundly impacting healthcare settings, signifies a mounting burden on healthcare infrastructure and resources. Accommodating this demographic transition necessitates a robust healthcare framework capable of managing chronic diseases, multiple comorbidities, and increasing surgical care in patients with frailty. Consequently, healthcare professionals, policymakers, and researchers are compelled to reconsider healthcare models, strategies, and policies to cater to the distinctive, complex needs of the aging population.

1.2 Advancements In Spinal Surgery

The field of spinal surgery has witnessed dramatic advancements in the past decade reflecting a promising trajectory in improving patient outcomes⁴. Spinal fusion, a surgical technique designed to join two or more adjacent vertebrae, has evolved significantly with the advent of cutting-edge technologies and innovative surgical practices. Modern fusion techniques are increasingly embracing minimally invasive methods, which mitigate surgical risks and speed up recovery times⁵. Furthermore, the development of novel biomaterials for bone grafting is revolutionizing fusion surgeries by improving bone healing potential and minimizing the need for bone grafting procedures. Perhaps one of the most transformative advancements has been the integration of computer-assisted navigation systems and robotics, offering unparalleled precision and safety during operations. These technologies enhance a surgeons' ability to navigate complex anatomical structures and conduct high-precision screw placement, minimizing the risk of nerve damage and postoperative complications⁴. Thus, the evolution of spinal fusion procedures underscores a broader trend of technological integration in spinal surgery, offering the potential for performing spinal surgery on patients previously deemed too frail or too old for such procedures.

1.3 Diagnosing Osteoporosis and Frailty

Diagnosing age-related conditions such as osteoporosis, sarcopenia, and frailty presents a unique set of challenges and implications within the healthcare sphere, specifically in relation to spinal surgery⁶. Osteoporosis, a disease characterized by low bone mass and structural deterioration of bone tissue, is typically diagnosed via Dual-Energy X-ray Absorptiometry (DEXA). This imaging technique measures bone mineral density, providing a comparative assessment of a patient's bone health relative to healthy individuals of the same age and sex. The condition predisposes individuals to an elevated risk of fractures and reduced bone healing potential, thereby increasing complication rates.

Sarcopenia, on the other hand, refers to the progressive and generalized loss of skeletal muscle mass and strength with advancing age. It is usually diagnosed using a combination of measures including muscle mass, strength, and physical performance, often requiring imaging techniques such as magnetic resonance imaging (MRI) or computed tomography (CT) scans, or bioelectrical impedance analysis (BIA). Frailty is a state of increased vulnerability to adverse health outcomes resulting from aging-associated decline in reserve and function across multiple physiologic systems. It is often identified via physical indicators such as unintentional weight loss, self-reported exhaustion, weakness, slow walking speed, and reduced physical activity. The co-existence of these conditions in a patient can significantly increase the complexity of spinal surgeries, as they are associated with higher rates of surgical complications, delayed recovery, and poorer surgical outcomes⁶. As a result, these conditions place considerable strain on healthcare systems due to the increased risk of disability, reduced quality of life, and the heightened demand for long-term care. Therefore, prompt diagnosis and effective management of osteoporosis, sarcopenia, and frailty are of paramount importance, not only for improving individual patient outcomes but also to alleviate strain on healthcare systems overall.

1.4 Biological Techniques to Increase Bone Healing

The role of biological agents in enhancing bone healing and spinal fusion rates is of paramount interest in orthopaedic and spinal surgery. One of the integral components of spinal fusion procedures is bone grafting. Significant efforts have been made to find alternatives to autologous bone grafting, the traditional gold standard graft material,

primarily due to limitations associated with donor site morbidity. Ideal graft materials should exhibit osteoinductive, osteoconductive, and osteogenic properties. Allogenic bone, which possess these properties, is a potential candidate but is limited by supply and the risk of immune rejection. Recombinant bone morphogenetic proteins (rhBMPs) were initially met with enthusiasm due to their ability to induce robust osteogenic differentiation. However, concerns surrounding their use and potential complication rates have arisen, tempering their application in the spinal surgery.

One of the drawbacks of synthetic bone graft materials, such as demineralised bone matrices and synthetic bone grafts, is the lack of a cellular or osteogenic component. This is where the use of mesenchymal stem/stromal cells (MSCs) comes into play. These cells, present in bone marrow, have the potential to differentiate into bone, cartilage, adipose, and several other tissue types. In spinal surgery, several clinical studies have employed MSCs in fusion procedures, often sourced from bone marrow aspirate (BMA). However, the low purity of MSCs in BMA has been a significant issue, prompting some clinical trials to use culture-expanded MSCs as a solution.

In recent years, the discovery of human skeletal stem cells (hSSCs) holds the potential to revolutionise the field of regenerative orthopaedic medicine. As multipotent, self-renewing cells, hSSCs are capable of differentiating into bone and cartilage, offering the potential to overcome the heterogeneity seen in the MSC population. This opens up the possibility of a more targeted approach to bone healing and spinal fusion, making use of these cells' specific capabilities for more effective treatment methods.

1.5 Thesis objectives

The objective of this thesis is to explore, from the hospital bedside to the laboratory bench, the current landscape of spinal fusion in Ireland, to explore potential novel tools to aid in the diagnosis of relevant age-related conditions and to explore the potential therapeutic potential of the hSSC.

Chapter 2 Anatomy and Applied Biology

2.1 Spine Anatomy

Understanding the anatomy of the spine and its related components is indispensable to decode the pathological alterations within the spinal column. The vertebral column, an intricate anatomical entity, is primarily constructed of vertebrae, intervertebral discs, ligaments, and the spinal cord. This bony vertebral column comprises 33 vertebrae, with 7 in the cervical region, 12 thoracic, 5 lumbar, 5 sacral (fused), and 4 coccygeal (fused) vertebrae⁷. Each vertebra typically includes an anterior load-bearing vertebral body with posterior facet joints, which articulate with the neighbouring vertebrae above and below. Despite a general similarity in structure, the vertebrae's anatomy within each region shows substantial variation, which enables the unique biomechanical functionality of each region.

The cervical spine is a mobile segment of vertebra allowing for movement of the neck. The range of motion permitted at the cervical spine is approximately 90 degrees of forward flexion, 70 degrees of extension, 20 to 40 degrees of lateral flexion and 90 degrees of lateral rotation⁸. The first and second cervical vertebrae, also termed as the atlas and axis, respectively, have unique anatomy owing to the articulation of the atlas with the skull via the occipital condyles. According to Greek mythology, the Titan warrior Atlas was condemned to carry the globe by Zeus after losing a battle to the Olympians. Hence, the C1 vertebra was so named. The C1 vertebra is ring shaped and lacks a body or spinous process. It is composed of two dense lateral masses of cortical bone connected by anterior and posterior arches. The C2 vertebra, the axis, is so named as it works as a pivot for the C1 vertebra. The odontoid process projects upwards from the body of C2 where it articulates with the anterior arch of C1. It is held in position by the transverse ligament which traverses from one side of the C1 ring to the other along the posterior transverse groove of the odontoid process. This unique anatomy permits a high degree of rotation at the C1-C2 (atlanto-axial) joint, therefore allowing the head to rotate⁸.

The cervical spine, characterized by its mobility, facilitates neck movements. The neck's range of motion includes approximately 90 degrees of forward flexion, 70 degrees of extension, lateral flexion between 20 and 40 degrees, and 90 degrees of lateral rotation⁸. The first and second cervical vertebrae, termed the atlas and axis respectively, have distinct anatomy due to the atlas' articulation with the skull via the occipital condyles. The C1 vertebra, being ring-shaped, lacks a body or spinous process, consisting instead of two robust lateral masses of cortical bone connected by anterior and posterior

arches. The C2 vertebra or axis serves as a pivot for the C1 vertebra. The odontoid process extends upwards from the C2 body, articulating with the anterior arch of C1. Its positioning is secured by the transverse ligament, which extends across the C1 ring from one side to another along the odontoid process's posterior transverse groove. This specialized anatomy enables a high degree of rotation at the C1-C2 (atlanto-axial) joint, permitting the head to rotate⁸.

The subaxial cervical vertebrae, including C3 to C7, exhibit a more conventional vertebral anatomy, comprising a vertebral body, lamina, facet joints, and a spinous process. The vertebral body is the primary load-bearing component of the spinal column. Intervertebral discs connect the vertebral bodies across the spine anteriorly, with the bodies typically growing taller as you descend the spine to accommodate the progressively increasing axial load. The body is cylindrical and anteriorly convex, made up of a slender external shell of cortical bone filled internally with porous cancellous bone and bone marrow. The endplates of the vertebral body, indicating the junction with the intervertebral disc, consist of a concave-shaped, thick ring of cortical bone. The peripheral endplate is the most robust, while the central part facilitates the osmotic diffusion of nutrients to the neighbouring intervertebral discs.

The cervical transverse processes jut out laterally and encompass the transverse foramina from C1 to C6. These foramina accommodate the vertebral artery, and a pronounced nerve root groove on the anterior surface carries the nerve root exiting at the same level. The pedicles, dorsal-lateral projections of the vertebral body, bridge the vertebral body and the lateral masses. Unlike those in the thoracic spine, pedicles in the subaxial cervical spine are small, short, and oriented medially.

The lateral mass is a dorsal-lateral bony structure with the superior and inferior articular surfaces of the facet joints located at its ends. Given their coronal orientation, the cervical facet joints are synovial joints, which allow for a wide range of motion in the cervical spine as this orientation limits movement in extension only.

The thoracic spine, composed of 12 vertebrae, exhibits a kyphotic orientation, setting it apart from the lordotic arrangement of the cervical and lumbar spine. The thoracic vertebrae, reflecting typical vertebral morphology, can be subdivided into three distinct segments: the cervical-thoracic transition, the lumbar-thoracic transition, and the mid-section. Of clinical interest is the mid-zone (T3 – T9), marked by a narrow canal diameter and vital vascular supply⁹. The facet joints' orientation gradually shifts from the cervical spine's coronal orientation to the sagittal orientation of the lumbar spine as you

move caudally, thereby incrementally increasing resistance to rotational forces. The thoracic spine's movement is further restricted by its articulation with the rib cage.

The lumbar spine, carrying five lumbar vertebrae, is lordotic in form, with each vertebra exhibiting a typical structure of an anterior vertebral body linked to a posterior neural arch¹⁰. Similar to thoracic vertebrae, the pedicles originate from the body's posterolateral surface and connect posteriorly with a pair of laminae. However, the lamina is relatively thicker and more vertically oriented than in the thoracic or cervical spine¹⁰. There is also a laterally positioned transverse process, articulating facet joints, and a spinous process located posteriorly (Figure 2-1).

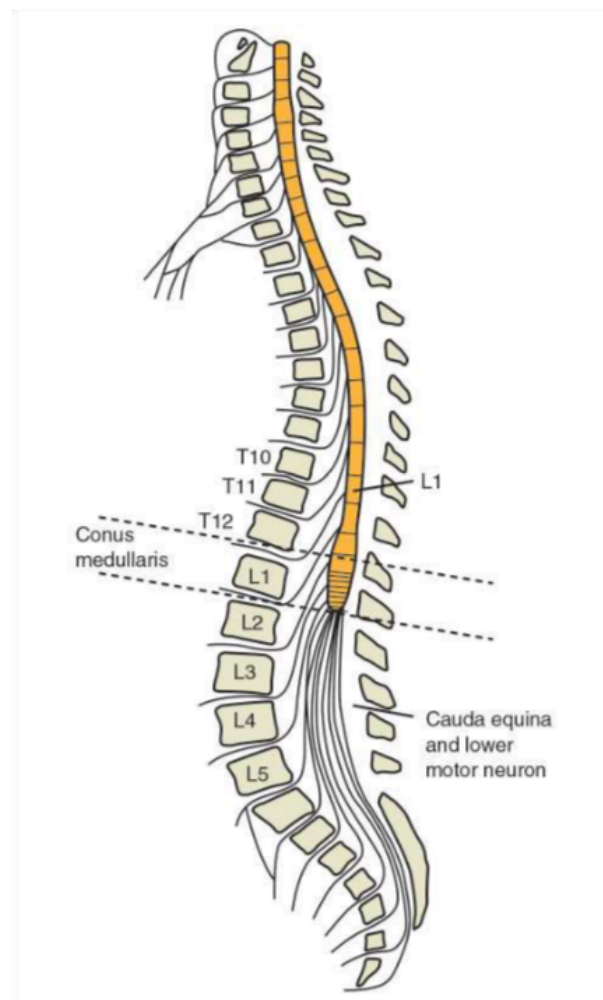


Figure 2-1 Schematic representation of spinal column

2.2 Tissues

2.2.1 Bone

Bone is a multifunctional, dynamic tissue of paramount importance to human physiology, offering structural support to soft tissues, facilitating locomotion, serving as a storage site for minerals, and participating in hematopoiesis¹¹. From a macroscopic perspective, bones are typically categorized into short, flat, and long or tubular based on their shape.

Vertebrae are categorized as short bones, although a vertebra's lamina could be viewed as a smaller flat bone. The bones of the cranial vault or the scapula provide more typical instances of flat bones. Mature bones house a central marrow area enveloped by bone tissue and an outer periosteum. Bone tissue comprises two types: spongy cancellous tissue and compact cortical bone, with cortical bone constituting 80% of the axial skeleton. While both cortical and cancellous bone share the same matrix composition, cancellous bone exhibits significantly higher porosity.

2.2.2 Intervertebral Discs

Intervertebral discs (IVDs) link each vertebra in the spine. The IVDs serve as a deformable substance, facilitating spinal flexibility and acting as a shock absorber against compressive forces. IVDs consist of three distinct tissue types: the inner nucleus pulposus (NP), the outer annulus fibrosis (AF), and the cartilage end plates (CEP)¹². The NP is a soft, hydrophilic substance situated at the disc's centre. The AF, emerging from the CEP, encompasses and contains the inner NP. IVDs anchor to their adjacent vertebrae via the cranial and cephalad rim of annulus fibrosis and the CEP¹². Found on the cranial and cephalad boundary of each disc, the CEPs are mature IVDs that lack blood vessels, relying on diffusion through the CEP with their adjacent vertebrae.

2.2.3 Ligaments

Ligaments, tough bands of connective tissue, serve the critical function of connecting two bones or cartilages, or securing a joint. Several vital ligaments fulfil these roles within the spine. The anterior longitudinal ligament (ALL) stretches from the basioccipital bone to the sacrum, traversing and attaching to the anterior border of the vertebral bodies and IVDs^{13,14}. This ligament broadens over the IVDs and becomes narrowest but thickest over the vertebral body¹⁵.

Conversely, the posterior longitudinal ligament (PLL) lays over the spinal canal's anterior aspect at the posterior border of the vertebral body and IVD¹³. It extends from the

C2 vertebra's body down to the sacrum. The PLL maintains a consistent width in the cervical and upper thoracic regions but adopts a denticulate, narrower form over the VB in the lower thoracic and lumbar regions¹⁵.

The ligamentum flavum forms the spinal canal's posterior boundary, running between the laminae along the spine's length. It attaches from the lamina above's deep surface to the upper edge of the lamina of the vertebra below¹⁶. The ligamentum flavum's lateral margin contributes to the facet joint capsule's anterior margin.

The interspinous ligament resides between the spinous processes of adjacent vertebrae. The anterior fibres arise from the ligamentum flavum and move upwards and backwards to attach to the inferior surface of the spinous process's anterior part. The midline fibres travel upwards and backwards from the inferior spinous process to the superior adjacent spinous process's inferior aspect¹⁷.

The supraspinous ligament extends posteriorly from the C7 vertebra's spinous process to the sacrum. However, there's some debate regarding the ligament's caudal endpoint in the lumbar spine as the fibres merge with the lumbodorsal muscles' insertional fibres^{15,18}.

Lastly, the intertransverse ligaments, found in the lumbar spine, connect one transverse process to the next^{17,18}.

2.2.4 Spinal Cord

The spinal cord carries both motor and sensory functions for the body. Motor function is regulated by a sequence of structures, including the cerebral cortex, internal capsule, corticospinal tracts, pyramidal tracts, and anterior horn cells¹⁹. It is estimated that 90% of the pyramidal tract crosses at the medulla to the contralateral lateral corticospinal fasciculus, where tracts controlling the upper extremities lie medial to those controlling the lower extremities.

Constructed of central grey matter and peripheral white matter, the spinal cord has distinct regions with different roles. The grey matter comprises efferent neuron cell bodies and houses different functions: the posterior horns oversee somatosensory function, the anterior horns manage somatomotor function, and the intermediolateral horns handle visceral function. Furthermore, the grey matter also serves as a hub for somatic reflexes.

White matter, composed of nerve fibres and glia, hosts different tracts: the posterior column contains the fasciculus cuneatus and fasciculus gracilis; the lateral column holds the descending motor lateral corticospinal and lateral spinothalamic fasciculi; and the anterior funiculus carries the ascending anterior spinothalamic tract and several descending tracts¹⁹.

The sensory function is controlled by the posterior columns, the lateral spinothalamic fasciculus, and the anterior spinothalamic fasciculus. Sensory tracts within the posterior columns carry responsibility for fine touch, proprioception, and vibration sense and cross over to the opposite side in the medulla oblongata on their path to the sensory cortex. Severing a posterior column results in loss of these sensory functions on the same side of the lesion. This stands in contrast to pain and temperature sensations, conveyed by the lateral spinothalamic tracts. These tracts cross within the spinal cord itself, leading to sensory loss on the opposite side to damage of one of these tracts¹⁹.

The spinal cord terminates at the L1 to L2 level, tapering to form the conus medullaris. The cord spans approximately 45cm in length, with an additional 25cm of filum terminale. It possesses an average diameter of 10mm and extends in length by about 10% upon flexion. Notably, the spinal cord has two enlarged regions – one to supply innervation to the upper extremities and another for the lower extremities¹⁹.

The vascular supply to the spinal cord is both intricate and crucial to its proper function. In the cervical region, the anterior and central aspects of the spinal cord are primarily supplied by the anterior spinal artery¹⁹. This artery arises from the vertebral arteries' two medullary feeder vessels near the brainstem.

In the thoracolumbar region, the spinal cord's vascular supply comes from the anterior spinal artery and two posterior spinal arteries²⁰. The superior intercostal artery, a feeder vessel of the deep cervical artery originating from the right subclavian artery, supplies the spinal cord with blood in the region of the cervicothoracic junction. The upper thoracic cord gets its somewhat fragile blood supply from several segmental vessels, with a particularly vulnerable zone (the "watershed critical zone") situated between the T4 and T10 levels. One dominant artery that plays a crucial role in this supply is the artery of Adamkiewicz. It arises from the left posterior intercostal artery at the T10 level in 80% of individuals, but it can originate anywhere from T5 to L5 in other cases²⁰.

The venous drainage of the spinal cord primarily relies on the azygous and hemiazygous venous systems. The veins of the spinal cord are located both anterior and

posterior to the cord itself. These veins drain into the extradural, valveless Batson venous plexus. This plexus has significant clinical implications as it is crucial in the aetiology of pyogenic spinal infections and the dissemination of metastatic disease.

2.2.5 Spinal Nerves

There are 31 pairs of spinal nerves - 8 in the cervical region, 12 in the thoracic region, 5 in the lumbar region, 5 in the sacrum, and 1 in the coccyx. The structure of each spinal root consists of motor and sensory rootlets, a dorsal root ganglion, and a spinal nerve. The sympathetic nervous system is supplied by myelinated preganglionic rami and unmyelinated postganglionic rami. Important branches of the spinal nerve include the sinuvertebral nerve, which supplies the anulus fibrosus of the intervertebral disc, and the dorsal ramus, which supplies the facet joints and posterior paraspinal musculature. The intervertebral disc in the lumbar region receives innervation from the sympathetic fibers anteriorly and the sinuvertebral nerve posteriorly. The sinuvertebral nerves innervate the posterior longitudinal ligament, the posterior portion of the anulus fibrosus, the ventral aspect of the dura mater, and the superior portion of the intervertebral disc. The dorsal primary rami give off medial, lateral, and, occasionally, intermediate branches. The facet joints, interspinous ligaments, and segmental muscles are supplied by the medial branch, with the iliocostalis and longissimus muscles supplied by the lateral and intermediate branches, respectively^{20,21}.

When considering the anatomic pathway of the spinal nerves, it is important to be aware of regional variations. In the cervical spine, the C1 nerve arises above the C1 vertebra, whereas the C8 nerve arises above the T1 vertebra. Conversely, in the thoracic and lumbar spine, the spinal nerve passes under the pedicle of the same-numbered vertebra. The spinal nerves emerge within the intervertebral foramina. In the cervical spine, no intervertebral foramina exist for C1 and C2; in the remaining subaxial spine, the C3 – C8 nerve roots emerge through the corresponding intervertebral foramina and occupy approximately 75% of their respective foramina. Thoracic nerves are much smaller, occupying only 20% of their foramen. Lumbar spinal nerves are larger, occupying approximately 33% of their respective pedicles. In the sacral region, the anterior and posterior rami exit the sacrum through their respective anterior and posterior foramina^{20,21}.

The human body houses 31 pairs of spinal nerves, which are segmented into various regions - 8 pairs in the cervical region, 12 in the thoracic, 5 in the lumbar, 5 in the sacral, and 1 pair in the coccyx. Each of these spinal nerves is made up of both motor and sensory rootlets, a dorsal root ganglion, and the spinal nerve itself.

The sympathetic nervous system receives its supply via myelinated preganglionic rami and unmyelinated postganglionic rami. Some notable branches of the spinal nerve are the sinuvertebral nerve, which supplies the anulus fibrosus of the IVD, and the dorsal ramus, which supplies the facet joints and posterior paraspinal musculature.

In the lumbar region, the IVD receives innervation from the sympathetic fibres at the front and the sinuvertebral nerve at the back. The sinuvertebral nerves innervate various structures, including the PLL, the posterior portion of the anulus fibrosus, the ventral aspect of the dura mater, and the superior part of the IVD. Meanwhile, the dorsal primary rami give rise to medial, lateral, and sometimes intermediate branches. The medial branch supplies the facet joints, interspinous ligaments, and segmental muscles. The iliocostalis and longissimus muscles receive their supply from the lateral and intermediate branches, respectively^{20,21}.

2.3 Skeletogenesis and bone formation, remodelling and repair

Bone serves a number of important functions; it acts as a scaffold and support for the body, a home for haematopoiesis and is an endocrine organ. The complexity of the skeleton is typified by the events controlling embryonic skeletal development and bone formation, remodelling and repair. Skeletogenesis, the formation of the skeletal architecture during embryonic development, is a complex process regulated by a finely tuned sequence of gene activation and cell recruitment²². There are two main phases of skeletogenesis: skeletal patterning and differentiation. In the first phase, skeletal patterning, mesenchymal cells converge at the sites where the skeletal elements will eventually be formed. In the second phase, differentiation of the various cell types which makeup bone occurs in a highly coordinated sequence of cellular proliferation and differentiation events. Bone formation, the final step in skeletogenesis, is dependent on vascular invasion²³ and can occur via either endochondral ossification or intramembranous ossification.

Endochondral ossification is a process whereby a cartilage template is formed in the first instance, followed by vascular invasion by bone cells. Chondrocytes are the first

skeleton-specific cell type during development, and they serve to facilitate bone formation via this process of endochondral ossification. In addition, they are necessary for longitudinal skeletal growth and are the primary cell type of joints, which permits mobility of the skeletal elements. Following skeletal patterning, mesenchymal cells produce an extracellular matrix (ECM) composed of type I collagen. The inner cells then differentiate into chondrocytes and produce an ECM composed of type II collagen and aggrecan, a proteoglycan²⁴. This proteoglycan serves to protect collagen fibrils, resist compressive forces and recruit growth factors such as fibroblast growth factors and hedgehog protein^{25,26}. The cells at the periphery of these condensations continue to produce type I collagen and form the perichondrium²⁷.

The skeletal microarchitecture is composed of three primary cell types. These are the bone-forming osteoblast, the bone-resorbing osteoclast and the osteocyte, a mechanosensor, an endocrine cell and the bone-lining cell.

2.4 Skeletal Stem Cells and Applications to Tissue Engineering/Regenerative Medicine

The fields of tissue engineering and/or regenerative medicine employ both engineering and biological principles to rebuild damaged and diseased tissues. These fields provide an exciting avenue for the treatment of a wide range of disorders, particularly musculoskeletal pathologies. Currently available options include platelet-rich plasma, purified cytokines, various matrix materials and cell-based therapy²⁸. Cell therapy involves the delivery of cells into a patient, via injection, grafting or implantation, to aid in the regeneration or repair of a damaged or diseased tissue. ‘Mesenchymal stem cells’ are a highly desired cell for these applications due to their inherent ability to self-renew and differentiate into multiple different cell types and have been the focus of considerable research efforts in recent decades²⁹. However, the commercial arm of these efforts has outpaced the supporting scientific evidence behind these therapies, resulting in numerous therapies marketed to patients despite supporting evidence²⁸. Already, these unproven, unlicensed products are causing patient harm and damaging the reputation of the field³⁰.

One of the principle factors at play limiting both scientific progression and the flourishing of these unproven therapies is the lack of a precise definition of a stem cell. This leads to both inconsistent and inadequate reporting of cellular preparations and the ability for companies to market cell preparations as MSC or stem cell therapies. The concept and term ‘mesenchymal stem cell’ dates back to 1991, where it was first

proposed by US biologist Arnold Caplan³¹. Over the ensuing decade, a vast number of papers detailing various tissue types from which MSCs could be isolated from and differentiated into were published³². The International Society for Cellular Therapy (ISCT) first sought to address this issue in 2005, stating that the term ‘multipotent mesenchymal stromal cells’ (MSCs) is the correct designation for the plastic-adherent cells from bone marrow (BM) and other tissues, that are often termed mesenchymal stem cells^{29,31,33}. Furthermore, the ISCT proposed a minimum set of defining criteria for MSCs which include 1) plastic adherence 2) specific surface antigen expression ($\geq 95\%$ of the MSC population must express CD 105, CD 73 and CD 90 and $\leq 2\%$ expression of CD 45, CD 34, CD 14 or CD11b, CD 79 α or CD 19 and HLA class II) and 3) multipotent (osteogenic, chondrogenic and adipogenic) differentiation potential under standard laboratory conditions²⁹.

Unfortunately, this position paper failed to quell the interchangeable use of the terms ‘mesenchymal stem cells’ and ‘mesenchymal stromal cells’ prompting a further position paper by the ISCT in 2019³⁴. Here, the ISCT reiterated their recommendation for the continued use of the term MSC and inclusion of MSC research under the ‘stem cell’ umbrella at scientific conferences, publications and funding bodies, to enable scientific continuity, discourse and progression. However, further tissue specific annotation is now required to detail the tissue of origin of MSCs due to the ‘varied phenotypic, functional and secretome behaviour’ of MSCs from different tissues³⁴⁻³⁶. A 2016 study determined that cell populations, all classed as ‘MSCs’, from various tissues exhibited considerably different genetic expression and differentiation capacity³⁷.

To eliminate the inappropriate use of the ‘mesenchymal stem cell’ term, the ISCT now recommends that there must be both *in vitro* and *in vivo* data to support the self-renewal and differentiation capacity of the cell preparation to use the term ‘stem’ cell. This is a considerable change from the 2005 guidelines as now colony fibroblast forming units (CFU-F) assays and *in vitro* trilineage potential assays are no longer sufficient to demonstrate self-renewal and differentiation capacity in the absence of *in vivo* data. Finally, the ISCT has placed considerable importance on the functional characterisation of the MSC preparation due to the recognised paracrine and immunomodulatory effect of MSCs, which have tissue specific characteristics and are the focus of number of potential MSC products³⁸.

It is now accepted that MSCs represent a heterogeneous mixture of cell types with varying lineage commitments and differentiation capacity. The idea of a single pluripotent stem cell present within the adult body is now in doubt and attention has since turned to search for lineage committed tissue resident stem cells. Recently, both Chan et al^{39,40} and Debnath et al⁴¹ have since reported on the identity of skeletal stem cells. In 2015, Chan et al first described the existence of a skeletal stem cell, and its progressively more committed downstream progeny, present in mouse (mSSC) that was capable of self-renewal and differentiation into bone, cartilage and stroma³⁹. Expanding on this work in 2018, Chan et al reported on the human counterpart – the human Skeletal Stem Cell (hSSC), again capable of self-renewing and differentiating into bone, cartilage and stroma, but not fat⁴⁰. This cell was found to be present in both foetal and adult long bones, in BMP-2 (bone morphogenic protein-2) treated adipose stroma and induced pluripotent stem cells. Moreover, it was shown to undergo expansion in response to acute skeletal injury. Both the hSSC and mSSC are identified with a distinct set of cell surface markers. Debnath et al described the presence of a periosteal skeletal stem cell present in mouse, capable of self-renewal, trilineage potential and which formed bone primarily through an intramembranous ossification mechanism⁴¹.

Chapter 3 Spine Surgery, History and Techniques

3.1 Spinal Fusion

Spinal fusion is a surgical technique whereby two or more adjacent vertebrae are manipulated to grow into a single solid bone. During a fusion (or arthrodesis) procedure, opposing bone surfaces of a joint are roughened and typically packed with bone graft material. This induces bridging bone to form across the gap, fusing the bones into a single unit⁴². Today, indications for spinal fusion procedures are wide and varied. These include, but are not limited to, spinal trauma, degenerative spinal conditions, spinal deformities (such as scoliosis), and tumour resection. Spinal fusion may be performed on any aspect of the spine, from the occiput to the sacrum. As techniques and technology have evolved in recent decades, so too have the indications for spinal fusion. As our knowledge of spinal instability and alignment has grown, in addition to superior diagnostic methods, we can now more precisely identify those patients who will do well with fusion procedures. The increase in navigation technology, including robotic assistance, has also lowered the threshold for fusion procedures in more unwell or comorbid patients.

The earliest reports of spinal column treatments date back to Egyptian times, whereby fractured cervical vertebra with concurrent paralysis was treated by rest with the additional application of bandages and dressings on wounds⁴³. Hippocrates (460-377 B.C.) described physical techniques to treat fractures without an associated paralysis, to prevent patients developing a kyphotic deformity⁴⁴. The first reports of spinal surgical intervention date to the late 18th century whereby Sir Percival Pott (1713-1788) described good results opening tuberculous abscesses and flushing with antiseptic solutions in 1778⁴⁵. Infections likely constituted the main obstacle to the progression of spinal surgery until the pioneering antiseptic work of Lister (1882 – 1912) and Semmelweis (1818 – 1865), lead to an increase in the total number of surgeries being performed⁴⁶⁻⁴⁸. The advent of general anaesthesia in 1844 by Wells who described narcosis induced by laughing gas, further contributed to the emergence of the spinal surgery field. From this point on, various procedures on both the cervical and thoracolumbar spine including both anterior and posterior approaches were developed. However, it was not until 1953 that Holdsworth and Hardy performed the first treatment with a *fixateur interne*. Harrington proposed his dorsal instrumentation system for the correction of scoliosis in 1958 and the first transpedicular screw fixation was performed in 1959 by Boucher. In 1964, Dwyer performed an anterior correction of scoliosis, however insufficiencies of the screw-and-rope system lead to pseudarthrosis, ultimately limiting its use⁴⁹. Zielke (1975), the Harms

Moss System (1991), and Kaneda (1991) include several subsequent modifications of the system that were developed⁵⁰⁻⁵².

With the advent of modern day technology, the growth of spinal surgery has accelerated rapidly. Between 2004 to 2015, there were approximately 2.8 million thoracolumbar, lumbar and lumbosacral fusion procedures in the United States for degenerative spinal conditions⁵³. Over this time, the volume of lumbar spinal fusion procedures increased by 63% in 2015 compared to 2004 (60.4 per 100,000 in 2004 to 79.8 per 100,000 in 2015). There was a concomitant increase of 138% in the volume of elective lumbar fusions in those over 65. Hospital costs too rose over this period, with the value exceeding \$10 billion in 2015 (from \$3.7 billion in 2004), with mean costs in excess of \$50,000 per admission in this patient cohort. Similar trends are seen in Europe, although it is more difficult to ascertain specific figures. Spine registries have been established in a number of European countries, in particular Sweden, Norway and Denmark, which give good insight into spinal surgery epidemiology. The Swedish Spine Registry, established in 1993, was the first European structured spine registry which aimed to cover disorders of the lumbar spine⁵⁴. According to the 2018 Annual Report, 9,484 patients underwent lumbar spine surgery in Sweden in 2017, and there has been a persistent increase in those undergoing lumbar spinal surgery since the registry's inception⁵⁵. Spine Tango was the first international spine registry, first representing 25 centres in 9 countries⁵⁶. However, this dataset is limited as no country is represented with a sufficient number of participating centres. Therefore, the exact incidence of spinal surgery per country is unknown. Furthermore, due to European data protection laws, inputted data is anonymised, thus limiting patient follow-up.

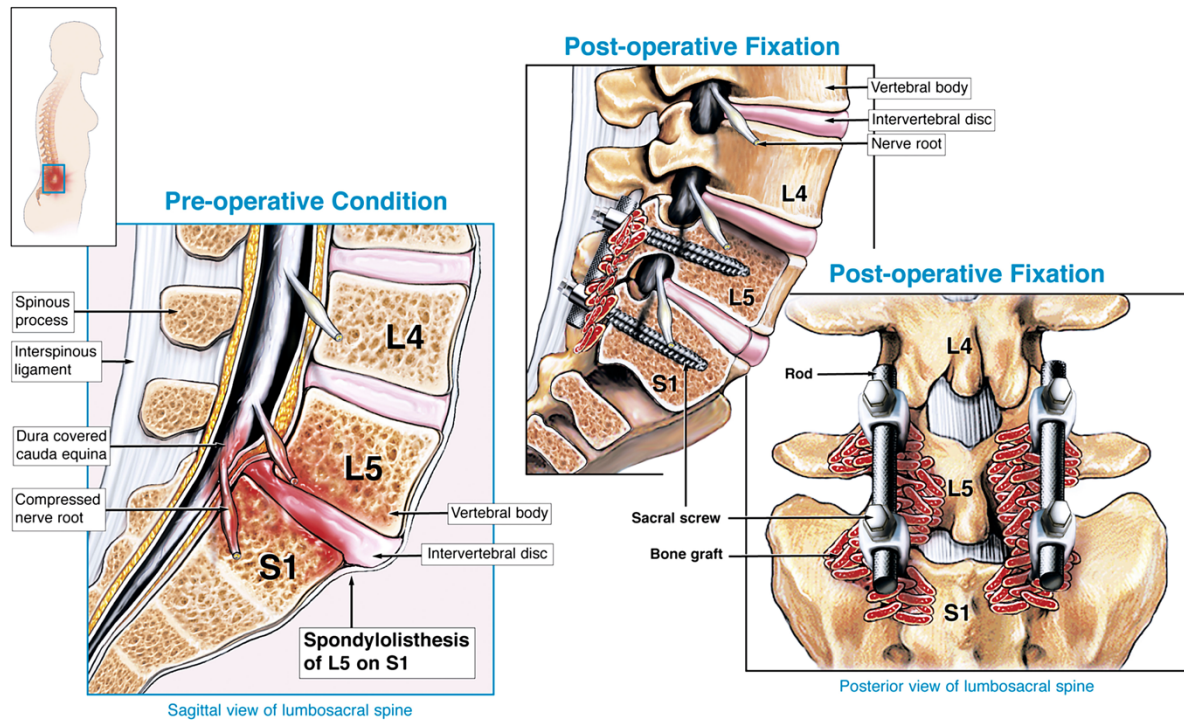


Figure 3-1 Diagrammatic representation of lumbar spine pre- and postoperative instrumented spinal fusion. Reproduced with permission from Alamy

3.2 Complications associated with spinal fusion

In comparison to other spinal procedures, spinal fusion requires the decortication of spinal joints and the placement of implants Figure 3-1. This often necessitates more extensive dissection and longer operating times. Therefore, it is not surprising that spinal fusion procedures have been reported to have twice the incidence of complications as non-fusion related procedures⁵⁷. These complications include an increased risk blood transfusion, donor site pain from iliac crest autograft harvesting, prolonged length of stay and mortality⁵⁷.

Failed solid bony fusion, or pseudarthrosis, is one of the most common complications of spinal fusion⁵⁸. The overall incidence of pseudarthrosis today is estimated to be 10-15%, and has been reported in up to 35% in some series, despite advances in surgical technology⁵⁹⁻⁶³. Establishing the clinical diagnosis may be challenging, as the symptoms may represent a number of diagnoses in the postoperative spinal patient. Patients typically report recurrent pain and/or neurological symptoms⁵⁸. Mechanical exacerbation of symptoms may point towards instability at the fusion site. The physical examination, likewise, is generally nonspecific, but may demonstrate spinal deformity due to loss of anatomical correction and alignment⁶⁴.

Traditionally, the gold standard for establishing a definitive diagnosis of pseudarthrosis is open surgical exploration to enable direct visualisation of the fusion mass. However, several imaging modalities are employed in the preoperative evaluation of suspected pseudarthrosis including plain radiography, computed tomography, and nuclear medicine investigations such as positron-emission tomography (PET)⁶⁵⁻⁶⁷. Heggeness and Esses classified pseudarthrosis into one of four categories: atrophic, transverse, shingle and complex⁶⁸. However, this classification system has yet to demonstrate clinical relevance⁶⁴. Radiographic pseudarthrosis may be present in the absence of symptoms. In the short term, radiographic pseudarthrosis typically has no impact on patient outcomes; however, several long-term studies demonstrate inferior patient outcomes and greater requirement for revision surgery in these patients⁶⁹⁻⁷¹.

Several surgical strategies are available for the operative management of pseudarthrosis. One option includes regrafting with or without revised instrumentation. Zdeblick demonstrated increased bony fusion rates in patients receiving autogenous bone graft with a rigid pedicle screw/rod fixation versus bone graft alone in those in posterior lumbosacral fusion⁷². Interbody fusion techniques offer promise, in terms of both treatment as well as prevention of pseudarthrosis. The interbody device confers an increased area under compressive force, thus providing a superior environment for fusion to occur. Potter et al⁷³ in their series of 100 patients undergoing transforaminal interbody fusion (TLIF) reported a radiographic fusion rate of 93%.

Given the complexity and potential severity of pseudarthrosis, a multifaceted approach to treatment is often required. As surgical technologies and techniques continue to evolve, ongoing research and long-term studies will be crucial in refining these approaches, ultimately leading to improved outcomes for patients affected by this challenging complication. Therefore, careful preoperative assessment, appropriate surgical planning, and the use of advanced fusion techniques remain integral to the successful management of pseudarthrosis.

Chapter 4 Image Guidance, Robotics, and the Future of Spine Surgery: A Narrative Review

4.1 Introduction

There have been considerable advances in spine surgery over the past number of decades⁷⁴. Image-guided navigation and robotic-assisted surgery are technologies that have progressed considerably and have potential to reduce complications and improve outcomes. These technologies can make a major impact on all areas of spinal surgery, such as minimally invasive spinal surgery (MISS), deformity correction, tumour resection, and revision surgery. These technologies typically require considerable financial investment and in the early adoption phase may be associated with potential disastrous operative complications. For these reasons, it is imperative that they are proven superior and safe before their mainstream use is adopted.

Robotic assistance offers the potential to mitigate fatigue and may facilitate superior precision of fine movement, irrespective of the length of the surgical procedure⁷⁵. This is desirable in a field such as spinal surgery, where procedures are often prolonged, physically taxing and demand delicate fine motor control throughout. Furthermore, the radiopaque and rigid nature of the adult axial skeleton offers great potential for the utilization of image-guided navigation and robotic assistance⁷⁶. The ever-increasing interest in achieving such precision and consistency is evident from the growing body of literature in the field of spinal robotics and the number of platforms coming to market⁷⁷. This review will give an overview of the most popular platforms available in both image-guided navigation and robotic assistance and provide an insight into the emerging technologies likely to impact spine surgery in the near future.

4.2 Navigation Systems

Navigation systems currently determine an instrument's location in space and in relation to their surroundings. Modern surgical navigation systems use a stereoscopic camera and reference markers attached to the patient in combination with marked instruments to determine instrument location and trajectories⁷⁸. This information is relayed to a monitor in the operating theatre allowing real-time feedback. The following include some of the more commonly utilized navigation systems currently available:

4.2.1 Airo Mobile Intraoperative Computed Tomography

Airo (Brainlab, Munich, Germany) is a circular scanner, attached to an operating room table, providing a full 360-degree CT scan (Figure 4-1). Instruments have 3 attached reference points recognized by a scanning stereotactic camera. The main advantages of this system are the slim gantry (30.5 cm×38 cm) with a large gantry opening (107cm), and the mobility of the scanner and table. For navigation purposes, instruments may be calibrated during anaesthesia, preparation and draping or exposure. An anatomic reference clamp is attached to a spinous process, allowing registration of the CT imaging. For minimally invasive procedures, 2 reference pins are placed in the iliac crests. A full 32-slice CT scan is obtained and the imaging is registered to Brainlab software. A real-time 3D map is then registered with precalibrated instruments, allowing stereotactic



Figure 4-1 Airo Mobile computed tomography. Image adapted from: www.brainlab.com/about-brainlab/image-gallery/

guidance of instrumentation.

4.2.2 O-Arm and StealthStation

The O-Arm and StealthStation S8 navigation system, by Medtronic (Minnesota, USA) involves a 360-degree CT-like scanner that can function as a 2D fluoroscopy device or

collect 3D volumetric imaging data. It relies on a scanning camera for instrument registration and allows for real-time instrument navigation and screw projection. Reference Clamps are placed on spinal processes and must not be touched, as it can alter the registration process thus leading to errors in stereotaxis. This causes the “map” generated to be shifted relative to instruments in space. If this occurs the patient will need to be rescanned.

4.2.3 Ziehm Vision FD Vario 3D

The Ziehm Vision FD Vario 3D (Ziehm Imaging, Nuremberg, Germany), is a C-arm based technology with the ability for 2D and 3D reconstructions (Figure 4-2). The 3D CT-like reconstructions can be combined for use with other navigation systems (such as Brainlab, Stryker, Medtronic). It also relies on a scanning camera for image registration and real-time navigation.



Figure 4-2 Ziehm Vision RFD 3D. Image adapted from: www.ziehm.com/en/products/c-arms-with-flat-panel-detector/ziehm-vision-rfd.html

4.2.4 Stryker SpineMask Tracker

Stryker's (Michigan, USA) SpineMask Tracker, attempts to negate the problem of reference point translation and allows the surgeon to work free of obstacles. It uses a flat rectangle of trackers, applied directly onto the skin of the patient's back. The system's camera connects with up to 31 tracking LEDs. It must maintain an unobstructed view of a minimum of 5 LEDs and furthermore, the surgeon's arm or body must not obstruct more

than half of the reference points of the rectangular tracker to allow accuracy of navigation. This system is limited by the size of the operative field, which cannot be extended beyond the boundary of the rectangular trackers, which is defined at the start of the procedure. Retraction or other movement of the skin may shift the trackers in relation to the axial skeleton, thus altering the navigation accuracy. This system is potentially better suited to minimally invasive procedures rather than more open procedures which demand more vigorous and dynamic retraction. (Figure 4-3)

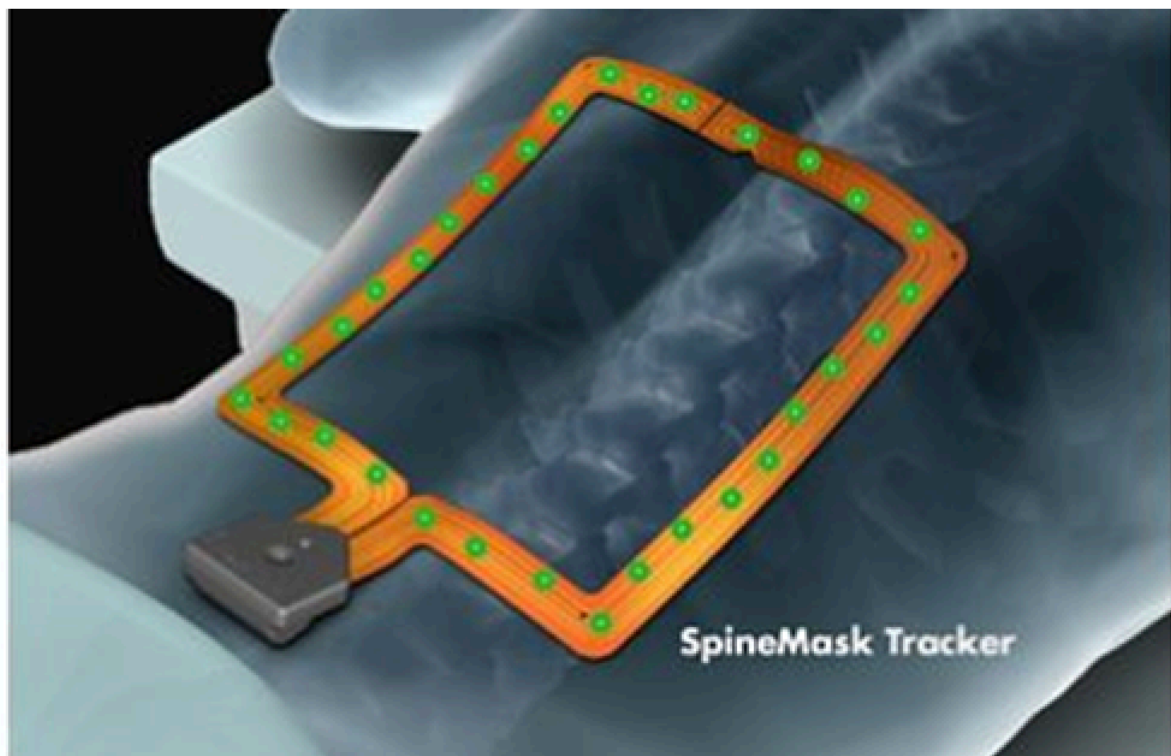


Figure 4-3 Stryker SpineMask® Tracker. Image obtained from <https://nse.stryker.com/products/spinemap-3d-software/>

4.3 Accuracy of Navigation Systems

Accurate placement of pedicle screws is essential to achieving a solid purchase for any spinal construct, as well as avoiding damage to structures in close proximity to the spinal column. Schwarzenbach et al⁷⁹ were the first group to examine the effect of computer-assisted navigation systems on pedicle screw placement. They demonstrated a breach rate of 2.7% in lumbar pedicle screws with the use of a navigation system. Numerous

meta-analyses and systematic reviews have been published in recent years examining the safety and accuracy of navigation-assisted pedicle screw placement versus free-hand placement⁸⁰. The primary endpoints utilized are typically accuracy of pedicle screw placement, radiation dose used, and operative time.

Overall, current evidence points towards increased accuracy in terms of screw placement with image-guided navigation systems⁸¹. Kotani et al⁸² demonstrated a lower breach rate and a corresponding improved safety profile in 109 cervical spine pedicle screw placement when computer assistance was used. Verma et al⁸³ performed the first 111 meta-analysis on this subject, published in 2010, reporting an improved accuracy with navigation-assisted pedicle screw placement. Although they found no neurological complication in those utilizing navigation (0/327 patients) and a neurological complication rate of 2.3% (13/569) in those undergoing free-hand placement, this failed to reach significance⁸³. Therefore, conclusions were unable to be drawn on whether this improved accuracy of pedicle screw placement results in improved outcomes for patients.

A number of reasons may explain why this accuracy did not readily translate into improved patient outcomes. First, free-hand pedicle screw insertion has an established high success rate⁸⁴. Aoude et al⁸⁵ in their systematic review found 91.4% of free-hand pedicle screws to be placed safely. Second, there exists the assumption that increased accuracy of pedicle screw placement will result in improved patient outcomes. However, studies published when pedicle screw placement was in its infancy in the 1990s reported free-hand pedicle screw misplacement rates as high as 20%–40%, yet this did not translate into increased complication rates^{86,87}. Gertzbein and Robbins⁸⁶ reported an accuracy of 81% of free-hand pedicle screws placed but without significant complications, a study that included only thoracolumbar pedicle screws. Although there is a relative paucity of studies reporting patient outcomes, recent studies failed to show decreased complication rates in those undergoing navigation assistance⁸⁰.

One difficulty when evaluating this topic is the lack of consensus on grading systems for pedicle screw accuracy⁸⁵. Many use 2-mm increments as the unit of breach severity, however, there remains no standardized grading system which takes into account degree of breach, side of breach (lateral or medial cortex), location within the spinal column and correlation with clinical symptoms⁸⁵.

4.4 Radiation Exposure

Intraoperative fluoroscopy is an integral part of many spinal procedures, commonly utilized for localization and guidance of instrumentation. Unfortunately, consequently, the ionizing radiation exposure is considerably higher in spinal surgery than in other subspecialties for all operating room staff⁸⁸. Furthermore, the increasing use of minimally invasive surgery, such as percutaneous pedicle screw placement and microdiscectomy, results in increased radiation exposure to the spinal surgeon^{89,90}. Rampersaud et al⁹¹ examined the ionizing radiation exposure to chest, thyroid, and hands during cadaveric lumbosacral pedicle screw placement. They found that spinal surgeons experience as much as 12 times greater exposure than nonspinal surgeons and are at a significantly increased risk of exceeding recommended annual dose limits. Bindal et al⁹² found that performing an unprotected minimally invasive transforaminal lumbar interbody fusion would reach annual occupational exposure limit after 194 cases, when averaging 1.38 levels per case.

The importance of lead protection cannot be over- emphasized during these fluoroscopically assisted cases. Mroz et al⁹⁰ found that the use of lead protection allowed the spinal surgeon to place up to 6396 screws before extremity exposure limits were met. Taher et al⁹³ echoed these findings in their study suggesting that 2700 lateral lumbar interbody fusion procedures can be performed per year before occupational exposure limits are exceeded if radiation safety guidelines are adhered to.

Computer-assisted navigation has the potential to further lower occupational exposure to ionizing radiation. Kim et al⁹⁴ demonstrated a significant decrease in radiation exposure to both patient and surgeon while using a navigation-assisted technique versus traditional fluoroscopy, without a significant increase in operative time. Kraus et al⁹⁵ found that radiation dose for fluoroscopy in posterior lumbar fusion far exceeded the dose for CT computer-assisted navigation registration, with navigation significantly reducing the radiation dose administered to the patient. Yu et al⁹⁶ in their systematic review echoed these results noting radiation exposure can be significantly reduced by the use of radiation protection and potentially with navigation-assistance technologies.

4.5 Robotic-Assisted Surgery

Advancement in technology has led to the refinement of navigation-assisted technologies, however, limitations still exist and accuracy is dependent on several variables as result of the interactions between the navigation platform, the operating environment and surgeon. Robotic-assisted technologies use the same navigation systems but have the advantages of avoiding the surgeon interfering with the tracking systems and decreasing the risk of human error through fatigue. Robotic assistance can be compartmentalized into 3 categories based on the level of assistance provided. First, there is the telesurgical systems, which have remote command stations whereby the surgeon controls every movement of the machine. Second, there is the “shared-control” model where both the surgeon and robot control motions. Lastly, there is the supervised controlled systems whereby the robot performs actions autonomously under close supervision of the surgeon⁹⁷.

4.5.1 SpineAssist / Renaissance / Mazor X (Mazor Robotics)

Mazor have been the pioneers in spinal robotics and their products are the most studied in the field⁹⁸. Their system operates under a shared-control model, with the pedicle drilling operation being performed by the surgeon. Preoperative or intraoperative surgical planning is possible with the assisted navigation system, which allows production of a template for screw entry point, size, and trajectory. The robot is mounted to the patient, at the spinal process in the open procedure or with a frame triangulated with K-wires for MISS. This eliminates relative motion between the patient and the robot. A short verification procedure using tracked K-wires in the mounted robot and the virtual template ensures accuracy of the procedure (within 1.5 mm).

The accuracy of this platform was confirmed in cadaveric studies, reporting accuracies within 1 mm of the preoperative template^{99,100}. Kantelhardt et al¹⁰¹ demonstrated a 95% accuracy of robotic-assisted pedicle screws versus 92% or fluoroscopically guided screws in their retrospective study of 112 patients. Xiaobang et al¹⁰² reported on 102 patients and 1085 planned robotic-guided pedicle screws. In 95 patients, 960 screws were implanted with robotic assistance, with 98.9% accuracy. In the remaining patients, pedicle screws were manually placed due to technical problems, with poor registration and trajectory problems as the main reasons for conversion to traditional methods.

Thus far, the only study to demonstrate inferiority of robotic assistance is a randomized control trial published by Ringel et al¹⁰³. This study with 60 patients and 298 lumbosacral pedicle screws demonstrated a 93% accuracy with the conventional free-hand placement versus 85% accuracy of the robot. The authors suggest that robotic assistance was a potential source of inaccuracy, with instability of the K-wire attached to the spinous process leading to malposition of drill sleeves and drill cannula. They therefore, suggested superior fixation of the mounted robot. (Figure 4-4)



Figure 4-4 Mazor Robotics Mazor X System. Image obtained from <https://spinalnewsinternational.com/mazor-x-a-successful-blend-of-technology-and-manual-intervention/>

4.5.2 ROSA

The ROSA robot, by Zimmer Biomet (Montpellier, France), is a free-standing robotic surgical assistant with a floor-fixable base and a rigid robotic arm, which mitigates concerns of fixation strength to the patient. The robotic arm moves with the patient using tracking camera monitoring and percutaneously placed tracking pins to the patients' bony anatomy. Lonjon et al¹⁰⁴ demonstrated an improved accuracy of this technique (97.3% for robot-assisted pedicle screws versus 92% for free- 27 hand placed) in a study of 20 patients and 40 screws. It is suggested that this robotic platform would be best suited for 29 MISS and percutaneous procedures due to the improved robotic arm fixation.



Figure 4-5 ROSA® robot, by Zimmer Biomet. Image obtained from <http://www.orthospinenews.com/2016/05/19/medtech-announces-first-rosa-spine-surgery-in-spain/>

4.5.3 Da Vinci Surgical System

The Da Vinci Surgical System (Intuitive, California, USA) is a telerobotic platform which has gained widespread use across various specialties including general surgery, urology, and gynaecology¹⁰⁵. It consists of 2 units: the surgical console and the robotic arms. The surgical console/booth is equipped with 3D vision screens and portals for the surgeon to control the surgical instruments. It has the advantage of having high definition stereoscopic vision (magnification $\times 10$), tremor filtering, limitless wrist range of motion and improved surgeon ergonomics.

In terms of application to spinal surgery, this platform has been used in laparoscopic anterior lumbar interbody fusion (ALIF). However, despite early promise of laparoscopic ALIF, no advantage has been shown over conventional open ALIF in terms of length of stay (LOS), blood loss, or complication rates¹⁰⁶⁻¹⁰⁹. This platform has a steep learning curve leading to an increased operative time and the system is not in common use by spinal surgeons. Currently, it is not FDA approved for spinal instrumentation. (Figure 4-6)



Figure 4-6 The DaVinci Surgical System

4.5.4 Excelsius GPS

The Excelsius GPS (Globus Medics, Pennsylvania, USA) is a new system launched in the USA in 2017. It is a system which offers both navigation assistance and robotic guidance capabilities. This system offers integrated instrumentation, involves minimal set-up time and has the potential to lead to a more optimal spine workflow. In a cadaveric study, Vaccaro et al¹¹⁰ demonstrated improved accuracy and reduced surgical time with 0 radiation exposure in this robotic model compared with conventional minimally invasive techniques. (Figure 4-7)



Figure 4-7 ExcelsiusGPS® - Globus Medical. Image obtained from <http://www.globusmedical.com/ExcelsiusGPS/>

4.5.5 Cirq

Cirq is BrainLab's new Robotic Arm, awaiting FDA approval. It is a slender, lightweight robotic device which attaches onto the operating table side-rail. This allows for minimal set-up time and interference with the surrounding operating room equipment and staff. Grip sensors allow for adjustment of individual segments providing significant flexibility. Currently this device is compatible with Kick and Curve navigation platforms and intraoperative imaging platforms such as Airo Mobile CT and Ziehm 3D C-arm. Attachable modules allow for operation-specific assistance.

4.6 Cost-Effectiveness

There remains a paucity of literature pertaining to cost-effectiveness of robotic assistance in spinal surgery, without which there will remain a barrier to investment for both navigation and robotic technologies in spinal surgery centres. Robotic systems, irrespective of surgical specialty, have high fixed costs. These costs include the initial purchasing of the system, maintenance and instrument costs. Annual service contracts of certain robotic systems are in excess of US\$100,000. Furthermore, training costs and the learning curve associated with these technologies are significant. In order to stimulate and maintain investment, these systems must confer advantages in both surgical outcomes and hospital/operating room efficiency. Several factors such as operative room time, length of hospital stay and surgical revision rates are measurable outcomes which influence cost that may be offset by use of these technologies⁹⁷.

Operative room time is considerable proportionate to operating room costs. A cost-effective analysis in 2010 by Watkins and Gupta¹¹¹ estimated that operative room time was ~\$93 per minute. Therefore, it stands to reason that decreased operative length will translate to cost savings. Vaccaro et al¹¹⁰ reported significant time savings from a cadaveric study using the Excelsius robot comparing robot versus conventional MIS techniques. Length of hospital stay is also considered a surrogate marker for overall hospital costs. Hyun et al¹¹² in their randomized control trial comparing MIS robot assistance versus fluoroscopy-guided open lumbar fusions reported significantly less hospital LOS. Decreasing LOS not only reduces the hospital cost per admission, but also allows for increased patient turnover thereby improving overall hospital efficiency. Revision surgery is a significant expense as a result of a repeated hospital admission and an additional surgical operation which has potentially increased operative length and complexity compared with the index procedure. Schroder and Staartjes¹¹³ in their review noted significantly less revision surgical rates in those undergoing robotic-assisted MIS posterior and transverse lumbar interbody fusion versus navigated and freehand procedures. Therefore, although sparse, current evidence suggests that robotic-assisted procedures result in indirect cost savings through decreased operative time, LOS, and revision surgical rates. Further studies are required to compare if the investment cost of the robot technology is offset or improved by these savings.

4.7 Future Directions

The future for navigation and robotic-assisted spine surgery is full of promise. Current areas for development lie in enhanced preoperative planning, improved navigation to include navigation for rods and interbody devices, disc space preparation and decompression procedures, as well as integration of rigid retraction systems. Further technological advances are on the horizon. Philips' in 2017 announced their "augmented reality" (AR) operating suite or "hybrid OR" which aims to improve on existing navigation systems by combining images of patients' anatomy with 3-dimensional reconstructed images (Figure 4-8). Xvision-spine AR surgical navigation, by Augmedics, is a system under development, funded by the AO foundation (Figure 4-9). It utilizes a headpiece worn by the surgeon which incorporates the patients preoperative CT imaging to allow real-time navigation without having to look away from the patient. Reference

points on both the headpiece and on the patient allow for both patient and surgeon movement during navigation.

Cost-effectiveness must be proven to ensure widespread uptake of these technologies. This may inevitably occur with time as more systems become available ensuring competitive pricing, as well as with expanded applications such as in tumor and deformity surgery.



Figure 4-8 Example of Philips' hybrid operating room. <https://www.compelo.com/medical-devices/news/philips-develops-new-surgical-navigation-technology-for-image-guided-surgery-130117-5715695/>



Figure 4-9 Xvision-spine augmented reality surgical navigation by Augmedics. Image obtained from <https://www.augmedics.com/>

4.8 Author's Preferred Treatment

Robotic systems further advance the assistance provided by navigation platforms, offering the potential for greater mitigation of fatigue to the surgeon. They allow for accurate and efficient pedicle screw placement. At present, these platforms are demonstrating the greatest potential to improve spinal surgery outcomes. However, time will tell whether the AR platforms will prove dominant over the robotics-assistance systems.

4.9 Conclusions

In conclusion, spinal navigation systems and robotic assistance offer huge potential for improving modern spinal surgery. This developing technology has been demonstrated to be safe and efficacious for pedicle screw instrumentation. It offers the advantages of improved dexterity, tremor elimination, indefatigability, and image magnification. This field is rapidly growing, typified by the proposed AR systems, which are currently under development. It is important that this evolving technology demonstrates superiority when compared with traditional techniques before its acceptance and widespread use.

Chapter 5 Surgical Considerations in Spinal Surgery for the Osteoporotic Patient

Spinal fusion is a technique commonly used in spinal surgery whereby adjacent vertebral segments are fused together to eliminate intervertebral motion. Traditionally, the indications for spinal fusion included severe scoliosis, spinal tuberculosis and fractures⁴². Today, the application of spinal fusion has expanded considerably to include spondylosis (degenerative spinal changes), intervertebral disc disorders and spinal stenosis and the market value for spinal implants and devices is estimated to be greater than \$2 billion per year in the United States⁴². However, spinal fusion is associated with complications including failure to achieve a solid fusion mass (pseudarthrosis), instrument failure and increased complication rates compared to non-fusion procedures. Rates of pseudarthrosis are high, typically quoted as 15% in most series, though the incidence may be higher in those with fusion spanning greater than three level^{114,115}. Revision surgical procedures may be necessitated in those who develop pseudarthrosis with persistent or debilitating symptoms.

Osteoporosis has been recognised as a global health concern and is defined as a bone mineral density (BMD) 2.5 SD or more below the mean¹¹⁶. It is a systemic disease, characterised by deterioration of bone tissue microarchitecture, low bone mass and increased bone fragility¹¹⁷. Worldwide, it is estimated to affect over 200 million people. In the United States, it is estimated that 10.2 million or 10.3% of those over 50 years of age suffer from osteoporosis. A further 43.4 million are estimated to have low bone mass, at risk of developing osteoporosis¹¹⁸. Primary osteoporosis has a female predominance owing to decreased oestrogen levels in the postmenopausal period with 30% of all postmenopausal women in the United States and European Union estimated to have osteoporosis, with more than 40% of these predicted to suffer a fragility fracture in their lifetime¹¹⁹. Direct medical costs of fragility fractures secondary to osteoporosis are estimated to reach \$25 billion per year in the United States by 2025¹²⁰. In the European Union, direct costs are expected to rise from €32 billion per year in 2000 to €77 billion per year in 2050¹²¹. (Figure 5-1)

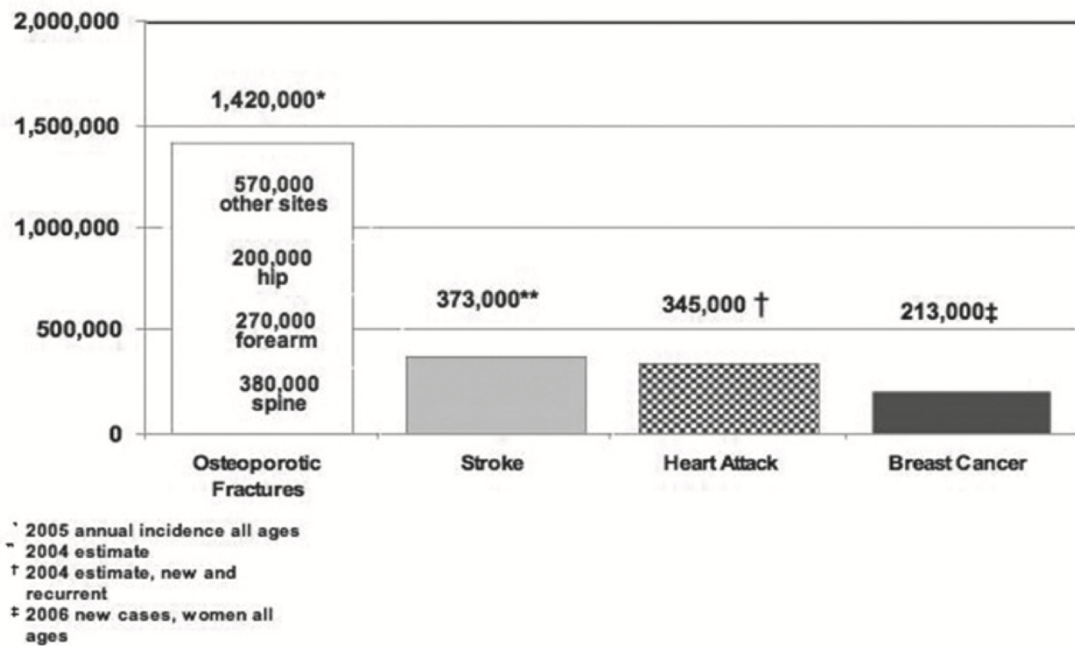


Figure 5-1 Comparative incidences of osteoporotic related fractures, new strokes, heart attacks and breast cancer in the United States. Adapted from Sozen T, Ozisik, L, Basaran NC, 2017. An overview and management of osteoporosis. *European Journal of Rheumatology*

Osteoporosis may be considered a syndrome of dysadaptation, leading to fragility fractures when bone formation and/or the response to strain is inadequate to provide sufficient strength for fracture resistance¹²². Osteoporotic fragility fractures typically manifest as distal radial, vertebral and femoral neck fractures. One fracture is a considerable risk factor for further fractures, with a reported incidence of 20% within one year following the index injury¹²³. Osteoporotic fragility fractures has been shown to adversely affect patients quality of life, and these effects have been shown to persist long after the index injury. Vertebral fractures, for example, may result in chronic back pain, loss of height and decreased physical function.

However, the clinical and economic burden of osteoporosis lies beyond the direct impact of fragility fractures. Lower back pain has a reported lifetime incidence of 84% and is commonly a result of degenerative spinal disorders such as spondylolisthesis, degenerative scoliosis and degenerative disc disease¹²⁴. As the mean population age rises, increasing numbers of these degenerative conditions are being diagnosed¹²⁵. If conservative, non-operative measures fail, surgical spinal fusion is the treatment of choice for these conditions which aims to eliminate painful intervertebral motion. Osteoporosis results in decreased fusion and remodelling rates due to decreased osteoblastic,

osteoconductive and osteoinductive capabilities¹²⁶. Pseudarthrosis is defined as the absence of solid bony fusion one year postoperatively and has a reported incidence of 15% in lumbar fusion procedures¹¹⁴. It typically manifests as persistent lower back pain with or without radicular pain as a result of continued motion¹²⁷. Revision surgical procedures may be necessitated in those who develop pseudarthrosis with persistent or debilitating symptoms. Several preoperative and intraoperative strategies exist to optimise spinal fusion rates. Intraoperatively, the use of bone graft is the current standard of care¹²⁸. Autologous bone graft remains the clinical ‘gold standard’ yet is limited due to availability and donor site morbidity. These limitations prompted the search for bone graft substitutes such as rhBMP-2 which have been proven to have superior fusion rates compared to iliac crest bone grafts^{129,130}. However, the use of rhBMP-2 is associated with potentially serious complications such as osteolysis, heterotrophic ossification, radiculitis, seroma formation, wound infection and malignancy¹³¹⁻¹³³. Current tissue engineering research aims to develop superior delivery vehicles to enable a smaller, more target dose of these compounds, as well as alternative materials¹³⁴.

Adjacent segment degeneration, proximal or distal to the fusion construct, is a potential complication of instrumented spinal fusion^{135,136}. Multiple factors contribute including intervertebral disc degeneration, compression fractures of the adjacent vertebrae, instrumentation failure, failure of the posterior soft tissue tension band weakened by surgical dissection¹³⁵. These factors are each more likely to be seen in an aging, osteoporotic patient cohort. This condition may present as back pain, or less commonly, with neurological deficits. Revision surgical corrections often require more extensive surgical undertakings (e.g. need for pedicle subtraction osteotomies) with extension of the fusion constructs¹³⁵.

In summary, the global burden of osteoporosis is considerable and continues to grow (Figure 5-2). As the life expectancy and average age of populations increase, so too does the incidence and prevalence of degenerative conditions such as those discussed above. Osteoporosis predisposes those undergoing spinal procedures to be at higher risk of suffering surgical complications, necessitating further, extensive revision procedures.

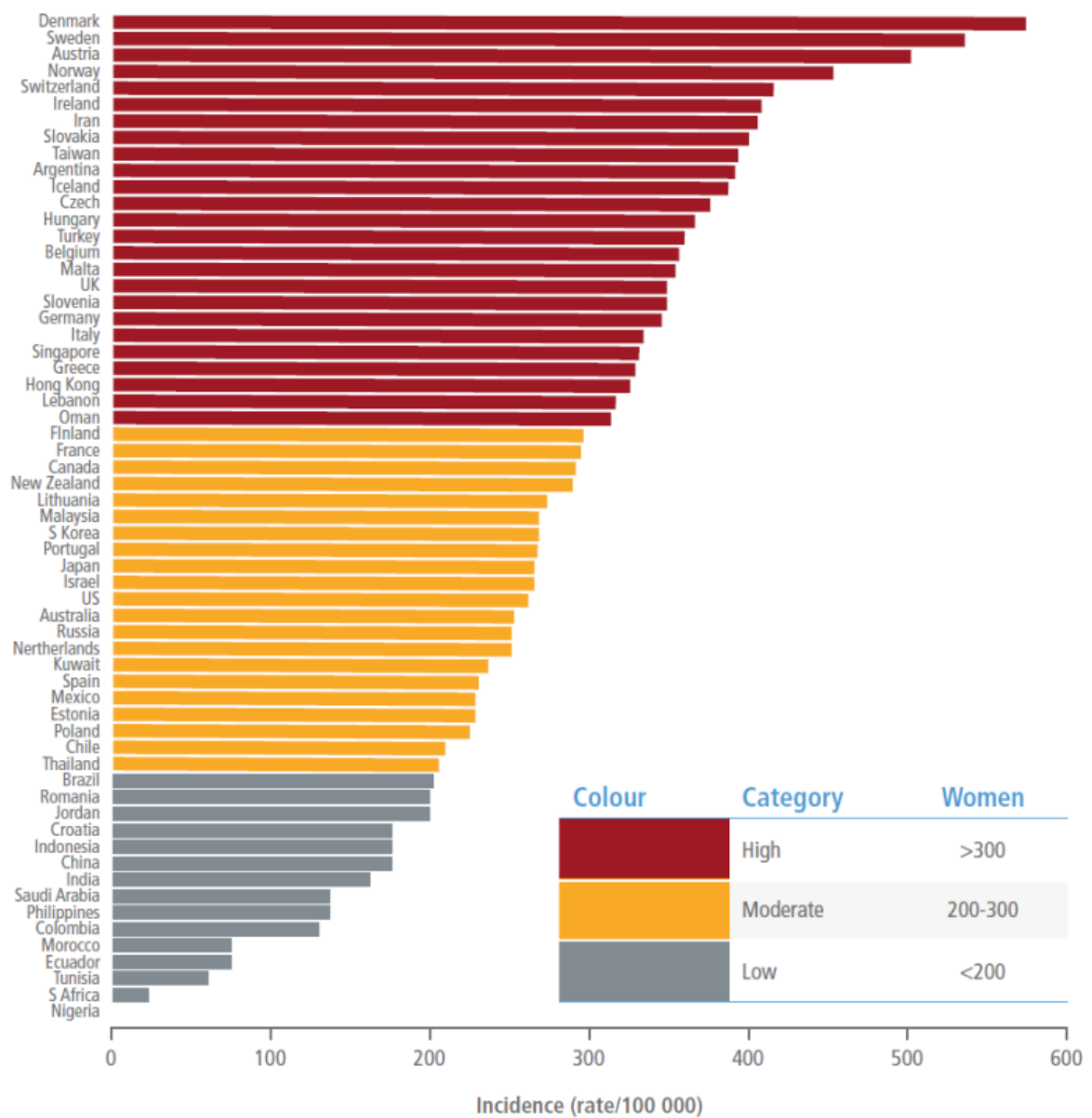


Figure 5-2 Age-standardised annual incidence of hip fractures in women (per 100 000) according to country, colour-coded as high, moderate or low incidence. Reproduced from Kanis et al., *Osteoporos Int*, 2012¹³⁷.

Chapter 6 Preoperative Considerations for the Frail Patient

6.1 Abstract

Frailty is a common geriatric syndrome, the relevance of which is becoming increasingly apparent in the clinical setting. It is often accompanied by varying degrees of sarcopenia and/or osteoporosis, leading to a decline in physical function, decreased levels of physical activity and overall poorer health outcomes in older adults. Identifying this cohort of patients prior to stressor events such as spinal surgery can prove paramount to improving the post-operative outcomes of these patients. This review provides a pertinent descriptive analysis to aid identification of frailty in a clinical setting, in addition to outlining methods of preoperative intervention that may improve postoperative outcomes.

6.2 Epidemiology and Aetiology:

Frailty is a common geriatric syndrome, the relevance of which is becoming increasingly apparent in the setting of spinal surgery. Characterised by a state of increased vulnerability, frailty results from age-associated declines in physiological reserve and function across multiple organ systems which compromises an individual's ability to cope with every day or acute stressors^{138,139}. The frail patient is more likely to suffer adverse events post-operatively, face longer hospital admissions, require further convalescence or assisted living facilities, and have increased mortality following spinal surgery^{6,140}. Frailty has been identified as an independent predictor of morbidity and mortality in spinal surgery¹⁴¹⁻¹⁴³. This increased likelihood of major complications has brought about an emerging area within spinal surgery which involves the risk stratification of patients not based solely on age but on their frailty status¹⁴¹. Optimisation of a patient's frailty status and efforts to increase their physiological reserve has the potential to improve outcomes in spinal surgery.

The two major models of frailty described in the literature include the *Frailty Phenotype* (FP)¹⁴⁴ and the *Frailty Index* (FI)¹⁴⁵. The FP uses the Fried Frailty Index (FFI) and defines frailty as a distinct clinical syndrome when three of five phenotypic criteria are met. These include weakness (grip strength), self-reported exhaustion, slow walking speed, low levels of physical activity, and unintentional weight loss¹⁴⁴. Alternatively, the FI considers frailty as an accumulation of deficits, providing a broader definition which includes both the physical and psychosocial aspects of frailty¹⁴⁶. A higher number of

health deficits indicates a higher frailty score. FFI and FI are both predictive of increased postoperative complications¹³⁸.

Roughly 8.5% of the global population is >65 years of age, and this percentage is set to increase to 16.7% by 2050¹⁴⁷. Due to a lack of consensus regarding the optimal diagnostic tool in defining frailty, there is, correspondingly, variation in reports of its prevalence in older populations with estimations in community dwelling adults ranging from 4-59.1%¹⁴⁸. The global prevalence of frailty is estimated to be 10.7%, with incidence rising to 26% in those aged over 85 years¹⁴⁸. Despite this and the possible implications of frailty for spinal surgery, little is known about the prevalence in the spinal surgery population¹⁴².

Sarcopenia represents another geriatric condition which commonly occurs in association with frailty⁶ and is considered a major physical driver of the frailty phenotype. Several definitions of sarcopenia have been proposed, however, no global consensus has been reached¹⁴⁹. The most commonly cited definition is that of the European Working Group on Sarcopenia in Older People (EWGSOP)), namely, a progressive and generalised skeletal muscle disorder characterised by systematic loss of skeletal muscle mass, strength, and function¹⁵⁰. Similar to frailty, reports on the prevalence of sarcopenia in the literature vary greatly, with figures ranging from 1-33% for different populations within the community¹⁵¹. Similarly, the incidence increases with age and is set to rise alongside the currently aging population¹⁵². This progressive skeletal muscle disorder is also associated with an increased likelihood of adverse outcomes secondary to falls, fractures, physical disability, and mortality¹⁵¹.

Osteoporosis, like sarcopenia, is a musculoskeletal disorder which predominantly affects older people. It is characterised by reduced bone mass and microarchitectural deterioration of bone tissue¹³⁹. Emerging studies have found frailty to be a promising predictor of osteoporotic fractures, suggesting there is a fundamental relationship between frailty and osteoporosis¹³⁹. Sarcopenia and osteoporosis are typical conditions seen in aging individuals; are often associated with each other and the frailty syndrome¹⁵³ and therefore, must be simultaneously considered when treating frailty in older adults¹⁵⁴.

No single underlying cause of frailty has been identified. Instead, the development of multisystem dysregulation - characteristic of frailty - is believed to be linked to pathophysiologic processes across numerous systems. These processes include chronic inflammation and immune activation, in addition to alterations in the musculoskeletal and endocrine systems¹⁴⁶. Inflammatory markers such as interleukin-6 (IL-6), C-reactive

protein and tumour necrosis factor alpha (TNF- α) have all been shown to be elevated in the frail population^{138,155,156}, in combination with a consistently elevated white cell count in frail patients¹⁵⁵. Chronic inflammation, in turn, can contribute to dysregulation of the musculoskeletal and endocrine systems which greatly affects skeletal muscle and bone mass, further perpetuating the development of frailty in older adults and acting as a key driver in sarcopenia and osteoporosis.

Muscle mass and strength peak in young adulthood and gradually decline as we age¹⁵⁷. Much like frailty, the development of sarcopenia involves several pathological processes. Between the ages of 25 and 80 it is estimated that a person loses roughly 40% of their muscle mass¹⁴⁹. This decline is associated with a reduction in the size and number of muscle fibres with preferential atrophy of type II fast-twitch fibres. Denervated motor units are subsequently reinnervated with slow motor units, leading to increased muscle fatigability and muscle weakness¹⁵⁸. Similar to frailty, sarcopenia has been associated with activation of pro-inflammatory cytokines, with studies demonstrating increased levels of TNF- α and IL-6 in skeletal muscle of aging adults^{158,159}. Molecular changes in sarcopenic muscle involve exacerbated age-related decline in hormones such as growth hormone (GH), testosterone, and insulin-like growth factor (IGF-1) (23) due to chronic inflammation, leading to loss of muscle mass and strength¹³⁸. As we age there is an increased deposition of adipocytes and fat infiltration of skeletal muscle^{157,160}.

6.3 Spinal Surgery:

Frailty, often accompanied by varying degrees of sarcopenia and/or osteoporosis, leads to a decline in physical function, decreased levels of physical activity and overall poorer health outcomes in older adults. Although an under researched area, frailty is also reported to have significant effects on morbidity and mortality in spinal surgery.

Flexman et al¹⁴² looked at a total of 53,145 patients with degenerative spine disease undergoing surgery. Flexman found that frailty was an independent predictor of major complications following spinal surgery with an odds ratio (OR) of 1.15 for every 0.1 increase in 11-mFI (95%CI 1.09–1.21, $p < .0005$)¹⁴². Ali et al identified 18,294 patients undergoing spinal surgery. They found that 8.1% of patients with a 11-mFI score of 0 (non-frail) suffered at least one complication post-surgery compared with 24.3% of patients with an 11-mFI score ≥ 0.27 (frail) suffering from at least one complication ($p < 0.001$)¹⁶¹. They also identified an increased risk of mortality post-surgery in frail patients.

An 11-mFI score ≥ 0.27 was associated with a mortality rate of 2.3% compared to 0.1% in patients with an 11-mFI score of 0 ($p < 0.001$)¹⁶¹.

Sarcopenia like frailty has significant effects on the outcome of spinal surgery. A study by Bokshan et al¹⁶², looked at the effects of sarcopenia on postoperative morbidity and mortality in 46 patients following thoracolumbar spinal surgery. They found that patients with sarcopenia had 3 times as many complications as non-frail patients (1.2 vs 0.4; $P=0.2$) and 10 times as many major complications following surgery (0.3 vs 0.03; $P=0.4$)¹⁶². Similarly, Eleswarapu et al¹⁶³, looked at 32 patients undergoing surgery for Adult Spinal Deformity (ASD) and found that patients with sarcopenia were more likely to suffer proximal junctional failure (PJF) and proximal junctional kyphosis (PJK), 37.5% and 62.5% respectively. Currently, a universal screening system for sarcopenia has yet to be developed however due to its implications for spinal surgery further research is required.

Due to these implications identifying this cohort of patients prior to spinal surgery is paramount to improving post-operative outcomes for these patients.

6.4 Clinical Presentation and Diagnosis

6.4.1 Frailty

There are many ways in which frailty in older adults can present itself. Common clinical signs of frailty include weight loss, sarcopenia, osteoporosis, a decline in physical activity, reduced gait speed, decline in cognitive function¹⁵³ and an increased number of falls. A growing number of frailty assessment tools have been developed, the majority of which are based on either the phenotypic frailty approach or the accumulated deficits approach¹⁶⁴. These frailty assessment instruments range from measuring a single item, such as grip strength to comprehensive evaluations with over 70 assessment parameters.

In a clinical setting, to be of use, assessments of frailty should ideally be quick and easy to perform. Based on this criteria, several simple screening tools have been developed and validated. Examples include Fried's Frailty Index (FFI), the Frailty Index of Accumulative Deficits (FI-CD), Clinical Frailty Scale (CFS), the FRAIL scale, and the Edmonton Frail Scale (EFS).

Fried's Frailty Index (FFI) is based on the phenotypic characterisation of frailty and defines frailty as the presence of three or more of: weakness (low grip strength), slowness (slow gait speed), low physical activity, exhaustion (self-reported) and unintentional weight loss of 4.5kg or more in the last year¹⁴⁶. FFI is widely used in the

clinical setting and is proven to be predictive of adverse clinical outcomes¹⁴⁶. Stemming from FFI, the *FRAIL scale* is a questionnaire composed of five components including Fatigue (Self-reported), Resistance, Ambulation (slow gait speed), Illness and Weight Loss (5% or more in the last year)¹⁶⁵. The patient is considered frail in the presence of three or more of these components¹⁴⁶. The FRAIL scale is a simple, cost effective questionnaire which can easily be performed in many clinical settings¹⁶⁶.

Frailty Index of Accumulative Deficits (FI-CD) assesses both physical and psychosocial aspects of the patient. FI-CD considers the accumulation of 30 or more comorbidities, disabilities, diseases, and symptoms. It is expressed as a ratio of deficits present, to the total number of deficits examined, a score exceeding 0.25 indicates frailty in a patient¹⁴⁶. FI-CD is generally considered a more sensitive predictor than FFI because it has a more finely graded risk scale and includes deficits which likely have a causal relationship with adverse outcomes, however, it is less commonly used in a clinical setting due to its time consuming nature¹⁴⁶. In attempts to combat this limitation, the Comprehensive Geriatric Assessment (CGA) can be used to complete FI-CD. CGA is used in geriatric settings to determine a patient's medical condition, mental health, functional status, and social circumstances. The FI-CD can be derived from data collected in a CGA which in turn decreases the time taken to complete¹⁴⁶.

Edmonton Frailty Scale (EFS) is a valid and reliable measurement of frailty often used in clinical settings¹⁶⁷. It evaluates 10 domains including Cognition, General Health Status, Self-reported Health status, Functional Independence, Social Support, Medication, Mood, Nutrition, Continence and Functional Performance¹⁶⁸, the scores of which are added together. The following cut-offs are applied to classify the severity of frailty: Not Frail (0-5), vulnerable (6-7), mildly frail (8-9), moderately frail (10-11) and severely frail (12-17)^{146,164}. Like other frailty assessments EFS can be derived from a CGA and takes little time to carry out¹⁴⁶. Clinimetric properties of discussed instruments and scoring systems are outlined in (Table 6-1).

Gait speed and Hand Grip Strength are alternative screening tools commonly employed. A 4m walking gait speed test can easily be performed in a clinical setting. Gait speed <0.8 m/s for both men and women is considered frail¹⁶⁹. Lower Hand Grip Strength scores act as a good predictor of adverse outcomes in older adults including increased functional limitations and longer hospital stays^{146,170,171}. Like gait speed, grip strength is also used in the assessment of frailty as it is predictive of muscle strength. Measurement

of grip strength is quick and simple, requiring only a hand-held dynamometer¹⁴⁶. The cut-off point for low grip strength is <27kg for men and <16kg for women¹⁵⁰.

Despite the identification of frailty as a significant predictor of morbidity and mortality in spinal surgery, there remains a lack of consensus regarding the most appropriate method of diagnosis¹⁴³. Further research must be undertaken to identify the most appropriate indices.

An important point to note is that although risk stratification of frail patients due to undergo spinal surgery provides invaluable information for clinicians, to date regardless of the index used there is a paucity of literature regarding the threshold at which a patient should be precluded from surgery and managed more conservatively. This is an area which requires future research.

Table 6-1 Clinimetric Properties of Certain Frailty Assessment Instruments

Instruments	Reliability			Validity				Other Attributes		
	Internal Consistency	Equivalence	Stability	Content Validity	Construct Validity	Criterion Validity	Cross-Sectional Validity	Sensitivity	Specificity	PPV/NPV
FFI					X	X	X			
FS	X	X		X	X	X	X	X	X	X
FI-CD		X	X	X	X	X	X	X		
EFS	X	X	X	X	X	X	X	X	X	

(FFI) = Fried Frailty Index. (FS) = FRAIL Scale. (FI-CD) = Frailty Index of Accumulative Deficits. (EFS) = The Edmonton Frailty Scale. (PPV) = Positive Predictive Value. (NPV) = Negative Predictive Value. (*) = data adapted from a recent systematic review by Faller et al. 2019.

6.4.2 Sarcopenia

Sarcopenia is often assessed alongside frailty as several of the physical performance markers used in the assessment of frailty, such as gait speed and hand grip strength, overlap with tests used in the diagnosis of sarcopenia. There have been numerous methods used within the literature to identify sarcopenia, however the optimal diagnostic algorithm has been debated¹⁵⁸.

Cruz et al suggests the use of a case finding approach in clinical practice¹⁵⁷. This involves investigating for sarcopenia when relevant symptoms are reported, such as increased falls, weakness, self-reported muscle wasting or weight loss, slow walking

speed and difficulty with activities of daily living^{151,157}. Case finding could be particularly useful in the setting of degenerative spinal disease where a high prevalence of sarcopenia might be expected.

The SARC-F has been recommended by the EWGSOP as a self-administered questionnaire that can be used to screen for sarcopenia¹⁵¹. There are five components of the SARC-F addressing strength, assistance with walking, rising from a chair, climbing stairs and falls¹⁷². Although the screening sensitivity of SARC-F is poor, its specificity is high and, therefore it serves as an effective tool in a clinical setting to identify patients who should undergo further diagnostic testing for sarcopenia¹⁷³.

The diagnosis of sarcopenia includes three parameters: muscle mass, muscle strength and physical performance^{151,157}. Muscle mass can be estimated by a variety of techniques, including Magnetic resonance imaging (MRI), Computed tomography (CT), Dual-energy X-ray absorptiometry (DXA) and Bioelectrical impedance analysis (BIA). As the optimal method of assessment is still widely debated, the choice of which of these to use relies largely on clinician preference and access to resources.

MRI and CT are considered the most accurate measure of muscle mass, however, are not widely used due to high costs and lack of portability¹⁵⁷. DXA is more commonly used and is currently the preferred method as bone mass can simultaneously be assessed¹⁵⁸, as outlined in Table 6-2. BIA works by estimating muscle mass based on whole-body electrical conductivity and represents a cost-effective, portable measurement tool which can be used at the bedside. A drawback of this method is that it uses population specific prediction equations¹⁵⁷, which the EWGSOP has recommended further studies are required to validate.

The evaluation of the other parameters of sarcopenia; muscle strength and physical performance, can be used to classify the severity of sarcopenia in patients with low muscle mass. The EWGSOP identified several tests which can be implemented in a clinical setting for this purpose, including hand grip strength, chair rise test, gait speed, timed-up-and-go test (TUG) and the short physical performance battery (SPPB) (Table 6-3).

Table 6-2 Diagnostic Parameters for Sarcopenia

Parameter	Assessment Tool

Muscle Mass	• MRI / CT (most accurate) • DXA (most commonly used)
Muscle Strength	• Hand Grip Strength • Chair Rise Test • Gait Speed • Timed-Up-and-Go-Test • Short Physical Performance Battery
Physical Performance	

(MRI) = Magnetic Resonance Imaging. (CT) = Computed Tomography. (DXA) = Dual Energy X-ray Absorptiometry

Table 6-3 Descriptive Characteristics of Assessment Tools for Muscle Strength and Physical Performance Used in the Diagnosis of Sarcopenia

Assessment Tool	Instructions	Clinical Utility
Hand Grip Strength	Requires the patient to squeeze a dynamometer	Quantifies maximum force or tension generated by a patient's grip
Chair Rise Test	Requires the patient to continuously rise and sit in chair (without the use of arms) for a pre-specified time period	Evaluates leg strength and endurance
Gait Speed	Requires the patient to walk a pre-specified distance on a level surface	Evaluates functional mobility and coordination
TUG Test	Patient is asked to rise from a chair and walk a pre-specified (usually 3.0m) distance and return to sitting	Identifies mobility or coordination issues
SPPB	A group of measures that combines gait speed, chair stand, and balance tests	Evaluation of lower extremity functionality

(TUG) = Timed-Up-and-Go-Test. (SPPB) = Short Physical Performance Battery.

6.4.3 Osteoporosis

Osteoporosis is diagnosed based on Bone Mineral Density (BMD). Osteoporosis is classified as a BMD of 2.5 standard deviations (SDs) or more below the mean of a young healthy adult woman ($T\text{-score} \leq -2.5$)¹³⁹. Fracture risk increases as BMD decreases. BMD can be measured using DEXA (gold standard) or Quantitative Computed Tomography (QCT). The accuracy of QCT in predicting spinal fractures is equivalent to DEXA with the added bonus that it measures volumetric BMD compared with areal BMD obtained by DEXA¹³⁹.

6.5 Interventions

As previously discussed, frailty is associated with worse outcomes in spinal surgery including increased morbidity and mortality^{141-143,161}. Could these complications potentially outweigh the benefits of surgical intervention? Risk stratification of patients based on their frailty status is only one component of the decision-making process. In order to effectively evaluate the use of such frailty indices we must understand the effect surgery has on the frail patient's quality of life (QoL). Reid et al, looked at Health-Related Quality of Life (HRQoL) improvement following surgery in Adult Spinal Deformity¹⁴¹. They found that despite a higher risk stratification and worse baseline HRQoL, frail patients were more likely to reach substantial clinical benefit for most HRQoL measures compared with non-frail patients¹⁴¹. The benefits of spinal surgery for such a cohort despite an increased risk of morbidity and mortality is a gap in the literature which has been recently identified in a systematic review by Chan et al, which highlights the need for further research regarding HRQoL outcomes following spinal surgery¹⁴³.

Once a frail patient has been identified and the decision to undergo spinal surgery has been made are there any interventions which can be implemented to improve a patient's frailty status, in turn improving surgical outcomes and QoL? To date there is a lack of research regarding specific intervention in the spinal surgery population however, we have identified some potentially promising options which could be implemented in such a population going forward, discussed below.

6.5.1 Frailty

Perioperative Approach

With respect to perioperative risk reduction, the benefits of establishing an individualised plan for frail patients utilising interdisciplinary shared decision-making to determine the

patient's deficits, the risks associated with the intervention and therapy goals, has been demonstrated.

Hall et al¹⁷⁴ used a Risk Analysis Index to screen for frailty in patients undergoing major elective non-cardiac surgery. Patients determined to be frail were flagged to the treating surgeon and multidisciplinary discussions were held with anaesthetists, critical care, and palliative care teams. Where indicated, perioperative plans were modified accordingly, and a reduction was observed in mortality among frail patients from 12.2% to 3.8% ($P < 0.001$). These findings suggest the efficacy of such initiatives in improving surgical outcomes.

Similarly, there is increasing evidence to support the value of Comprehensive geriatric assessment (CGA) (Figure 6-1) in the context of frailty. CGA is a multidisciplinary assessment¹⁷⁵ which aims to establish the medical, psychological, and functional capabilities of a frail older person so that a coordinated, integrated treatment plan for short- and longer-term follow-up can be developed.

In a surgical setting, the proactive care for older persons undergoing surgery (POPS) model has been shown to improve perioperative outcomes in older patients¹⁷⁶. Comparably, multiple meta-analyses have demonstrated significant reductions in post-operative mortality and rates of institutionalisation in patient populations who received CGA compared to standard care¹⁷⁷. Moreover, in the spinal surgical setting, the identification of these conditions will aid in surgical decision planning. In those with osteoporosis, the use of longer constructs and avoiding starting or ending a construct at junctional levels to prevent segmental or junctional failure when instrumenting the spine; pedicle screws can be undertapped to increase insertional torque and pull-out strength, in addition to the use of wider diameter cortical screws. There is an argument for the use of minimally invasive techniques, where possible, in those with sarcopenia to minimise disrupting the posterior tension band.

However, even without introducing the above-mentioned interdisciplinary meetings, simply being aware of a patient's frailty status may allow for more effective identification of possible risk factors and foster heightened vigilance for complications.

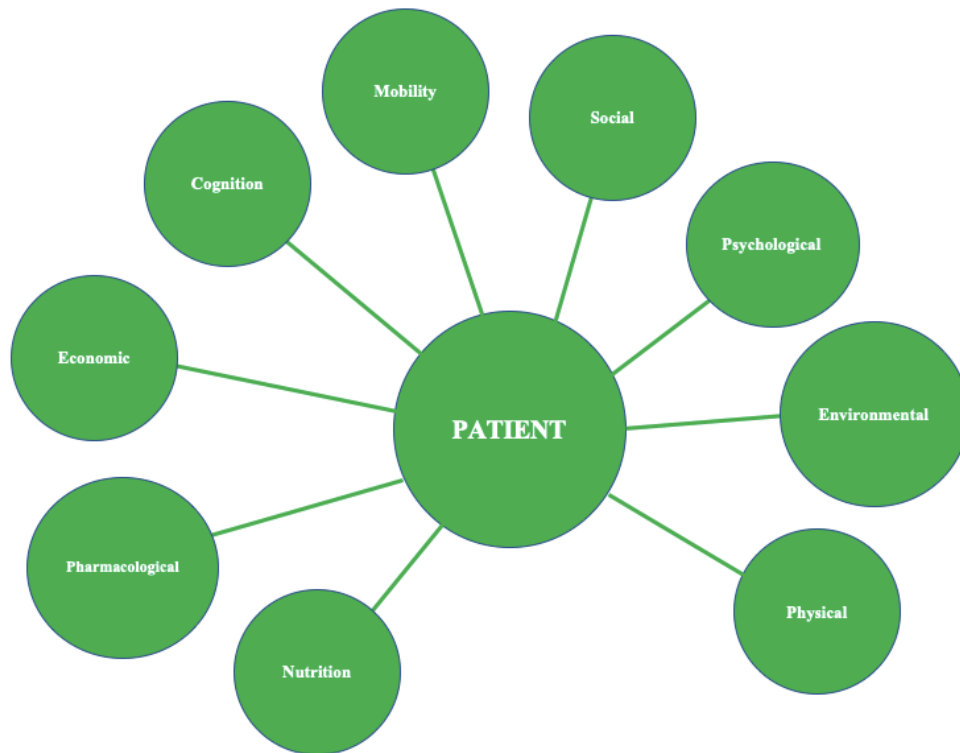


Figure 6-1 Components of the Comprehensive Geriatric Assessment (CGA). Adapted from Specialist Geriatric Team Guidance on Comprehensive Geriatric Assessment: National Clinical Programme for Older People (2016)

6.6 Interventions:

Identifying frailty during the preoperative assessment allows for patients to be pre-emptively enrolled in treatment programs which are aimed at enhancing an individual's functional capacity so they can better withstand the stress of the upcoming surgery. While, to date, no single evidence-based modifier of frailty has been determined, a range of non-operative interventions have been explored. This research has primarily focused on the effects of exercise and nutritional interventions, although, more recently, the viability of pharmacological agents in improving key measures of frailty is being increasingly explored.

Exercise is an attractive strategy and is known to have positive physiological impacts on several organ systems including immune, endocrine, and musculoskeletal, among others. Accordingly, several studies have demonstrated a positive impact on key measures of frailty including muscle mass and strength, as well as gait abilities¹⁷⁸. A systematic

review by Kidd et al¹⁷⁹ outlined the significant improvements that were observed in frail patients undertaking interventions, such as resistance training, which set out to improve physical condition. They also highlight that a combination of resistance and balance training was associated with improvements in treating the physical symptoms associated with frailty, while simultaneously reducing the risk of falls. These findings of the benefits of a multi-component exercise regime on measures of frailty have similarly been demonstrated elsewhere in the literature¹⁸⁰. Based on these findings, it is possible that an exercise intervention which consists of not only strength and resistance, but also flexibility and balance components may be most beneficial on improving key measures of frailty including mobility, muscle mass and strength, and activity.

It is worth noting that there is a lack of a standardised exercise protocol throughout the literature, and few studies directly evaluate the overall impact on a patient's frailty status.

While there is evidence to suggest a link between diet (both macro- and micronutrient intake) and the risk of developing frailty¹⁸¹, research in the area of nutritional supplementation as an intervention for frailty has yielded mixed results and evidence supporting its efficacy is lacking. Similarly, while the idea of there being a synergistic effect between nutritional interventions and exercise when used in combination does not seem unreasonable, the benefit of nutritional supplementation beyond exercise alone appears to be limited¹⁸².

Nonetheless, there are findings in the wider literature which suggest the importance of supplementation in malnourished individuals¹⁸³, and international guidelines which outline the use of nutritional interventions as a preventative measure to enhance recovery in older individuals at risk of malnutrition¹⁸⁴.

Many pharmacological strategies are currently under investigation with respect to improving measures of frailty, however none are currently adequately supported by the literature and remain important subjects for future research.

Hormonal replacements such as testosterone have been shown to have a positive effect on muscle mass and strength¹⁸⁵. However, their associated unfavourable systemic side effects reduce the attractiveness of such therapies in practice. Oestrogen replacement

therapy and growth hormones likewise have an unfortunate side effect profile and limited proven clinical efficacy thus far.

While there are systematic reviews to support the benefit of daily Vitamin D supplements (800-1000U/day) on gait and posture¹⁸⁴, generally studies have shown mixed results and thus there is lack of convincing evidence.

A potential role for angiotensin converting enzyme inhibitors (ACEi) in improving skeletal muscle has been proposed and there is currently ongoing research into their benefit in sarcopenia¹⁸⁶. However, again, their value in the context of frailty has yet to be extensively investigated. To date, ACEi have not been shown to significantly alter the incidence of frailty¹⁸⁷.

6.5.2 Sarcopenia

The potentially modifiable factors currently being targeted in sarcopenia include physical activity and nutrition. However, much as with frailty above, the heterogeneity between trials, in combination with the limited selection of randomised controlled trials, limits our ability to definitively establish their effectiveness.

Exercise is currently considered the most important strategy in the prevention and management of sarcopenia^{151,152}. In particular, the importance of resistance training has been repeatedly emphasised. Resistance training includes any physical activity which causes the muscles to contract against an external resistance. There is evidence from both systematic reviews and meta-analyses which links this form of exercise to improvements in muscle mass, muscle strength and physical performance in older individuals¹⁸⁸. These changes are thought to occur predominantly due to increases in the size and cross-sectional area of muscle fibres, with fast-twitch fibres (types IIa and IIX) in particularly implicated¹⁸⁹.

A systematic umbrella-review by Beckwee et al¹⁹⁰ recommends that for maximal strength gains, sarcopenic individuals should engage in a high-intensity resistance training program (i.e. 70-80% 1RM) with a focus on larger muscle groups. Between 1–4 sets of 8–15 repetitions per muscle group should be carried out 2–3 times per week. There may also be some benefit to a multimodal exercise program in the treatment of age-related sarcopenia, which consists of both aerobic and resistance exercises¹⁹¹.

There is a lack of robust evidence and large-scale trials into the role of nutritional supplementation in sarcopenia. Nonetheless nutrition, in combination with physical activity, is often raised as a strategy for preventing the declines associated with sarcopenia. There has been much focus on the role of protein supplementation. Past studies have demonstrated positive correlations between higher dietary protein intake and muscle mass and strength, greater bone mass density and slower rates of bone loss^{192,193}. Moreover, while older individuals require higher dietary protein than their younger counterparts¹⁹⁴, it has been found they often consume less protein¹⁹⁵. Bauer et al¹⁹⁴ have recommended an average daily intake of protein of at least 1.0-1.2 g/kg BW/d in older adults, with still higher amounts (1.2–1.5 g/kg BW/d) recommended in subpopulations such as those with an acute or chronic disease, or with marked malnutrition. In this context, protein supplementation is potentially an appealing strategy in preventing muscle loss.

6.5.3 Osteoporosis

The involvement of orthopaedic surgeons in treating patients with fragility fractures creates an opportunity to identify individuals with a high risk of osteoporosis¹⁹⁵, and thereby improve care by referring them for appropriate evaluation and treatment. Treatment of osteoporosis consists of both prevention strategies and of pharmacological therapies to address the patient's low bone mineral density.

As with frailty and sarcopenia, an emphasis should be placed on encouraging patients to participate in physical activity. More specifically weightbearing exercises such as walking and running, and resistance training appear beneficial in maintaining and improving bone strength, although the optimum frequency, duration and intensity still have yet to be determined¹⁹⁵. Introducing balance and muscle strengthening exercises may help reduce the incidence of falls.

Addressing diet is similarly important, particularly encouraging and ensuring adequate Calcium and Vitamin D intake. Recommendations by the National Osteoporosis Foundation suggest a total calcium intake of 1000 mg/day for men 50–70 years or 1200 mg/day for women over 50 and men over 70 years¹⁹⁵, and a vitamin D intake of 800–1000 IU/day. Dietary intake is preferred, however if insufficient, supplements should be incorporated. Other modifiable lifestyle factors to promote bone health include smoking cessation and reducing alcohol intake.

Decisions regarding pharmacological treatment mainly depend on the patient's risk of osteoporotic fractures. While management of lower-risk patients is generally limited to calcium and vitamin D, those at higher risk will require osteoporotic agents to improve bone mass and prevent fractures¹⁹⁵. These agents consist of antiresorptive drugs (typically bisphosphonates; denosumab; or, less commonly, the oestrogen receptor agonist ralocifene, or calcitonin) or anabolic drugs (such as the PTH receptor agonists teriparatide and abaloparatide) or those which act as a combination of both.

6.6 Authors Preferred Treatment

Regardless of the measurement index used it is clear from the literature that frailty is associated with a higher risk of morbidity and mortality in patients undergoing spinal surgery^{141-143,161}. Despite this it has been suggested that frail patients may achieve the most benefit from undergoing such surgery, with a significant increase in their QoL¹⁴¹. As a result, to improve outcomes following spinal surgery and potentially improve patient QoL further high-quality studies must be conducted to establish the most effective measure of frailty and a consensus reached regarding a universal measure of frailty in spinal surgery¹⁴³.

Once the diagnosis of frailty has been established, preoperative lifestyle changes aimed at improving functional reserve may help to reduce the risk of morbidity and mortality highlighted in the literature. Preliminary evidence for the value of multi-model exercise programs and protein supplementation is promising, notwithstanding the need for large-scale, high-quality randomised controlled trials to establish which protocol is most beneficial specifically in the spinal surgery population.

Chapter 7 Iliac Crest Bone Graft versus Cell-Based Grafts to Augment Spinal Fusion: A Systematic Review and Meta-Analysis

7.1 Abstract

7.1.1 Introduction

Despite successful fusion rates with iliac crest bone graft (ICBG), donor-site morbidity and increased operating time remain a considerable limitation and drive the search for alternatives. In this systematic review, cell-based grafts were compared with ICBG for spinal arthrodesis. We compared safety, efficacy and long-term outcomes, thus providing the current and relevant evidence for orthopaedic surgeons to make informed choices regarding this rapidly developing field.

7.1.2 Methods

An electronic literature search was conducted according to the PRISMA guidelines by two independent reviewers for articles published up to 1st March 2023 using PubMed, EMBASE and the Cochrane Central Register of Controlled Trial. The following data was extracted: number of patients, type of graft, fusion assessment method, follow-up duration, fusion rates, clinical outcomes and complications. The *Methodological Quality of Evidence (MQOE)* was assessed using the Risk Of Bias 2 (RoB-2) tool and Risk of Bias In Non-Randomised Studies (ROBINS) tool developed by Cochrane for evaluating bias in randomised and non-randomised studies.

7.1.3 Results

Ten studies fulfilled the inclusion criteria, including 465 patients. The mean number of patients per study was 43.8 (std dev. 28.81, range 12 – 100). Two studies demonstrated cell-based therapy to be significantly more successful in terms of fusion rates compared to ICBG. However, the remaining eight demonstrated equivocal results. No study found that cell-based therapy was inferior. No difference was seen between the two groups in three studies who focused on degenerative cohorts. No difference in functional outcome scores was seen between the groups. A number of different preparation techniques for cell-based grafts were used throughout the studies.

7.1.4 Conclusion

Cell-based therapy offers a promising alternative to ICBG in spinal fusion surgery, which could help reduce the associated morbidity to patients. This review found that cell based therapy is non-inferior to iliac crest bone graft and may offer patients an alternative treatment option with fewer complications and reduced post-operative pain. However, the literature to date is limited by heterogeneity of the cell preparation and grafting process. Future research with a unified approach to the cell preparation process is required to fully delineate the potential advantages of this technology.

7.2 Introduction

Spinal fusion (or arthrodesis) is a surgical technique used to surgically stabilise the spine and is indicated for a variety of pathologies including discogenic back pain, stenosis, radiculopathy, neurogenic claudication and degenerative spinal pathology¹⁹⁶. Fusion serves to stabilise pathological bone reducing pain, restoring lordosis and restoring physiological function¹⁹⁶.

First proposed in 1911, with the advent of improved surgical technology and techniques, the number of spinal fusion procedures performed has increased markedly in recent decades. In the United States, between the years 2004 and 2015, there was a 55% increase in the number of fusion procedures performed per annum¹⁹⁷. Successful arthrodesis is dependent on both patient and surgical factors. Patient factors include co-morbidities, medications, and smoking status with surgical factors including type of fusion technique, number of fusion levels and choice of bone graft. The reported overall success rate lies between 84% and 100%¹⁹⁸. Bone grafting remains a critical component of the fusion procedure with iliac crest bone graft (ICBG) being long been heralded as the “gold standard” graft. The posterior superior iliac spine is often favoured due to its greater abundance of potential bone graft available¹⁹⁹. However, harvesting ICBG has traditionally been associated with significant perioperative and postoperative morbidity. Chronic donor site pain is the most commonly reported complication, with a reported incidence ranging from 25 – 60%²⁰⁰. Other reported complications include deep infection, neurovascular damage, superficial infection, haematoma and seroma formation²⁰¹. This adverse effect profile of ICBG has prompted a vigorous search for alternatives in recent years. Cell based therapies (CBT) are one such avenue which has shown clinical promise that may provide this alternative.

Mesenchymal stromal cells (MSCs) offer great potential given their ability to differentiate into various cell types, most notably bone, cartilage and fat cells. In this way, bone marrow aspirate (BMA), a source of MSCs, can be harvested by percutaneous aspiration from the posterior iliac bone with minimal morbidity²⁰². Preparations of these CBTs can vary significantly, from “minimally manipulated” preparations derived from bone marrow to more onerous cell culture expanded cell preparations – a process which can take several weeks. Once the cells or sample of interest has been prepared, they are then transplanted to the area of interest, typically via a graft conduit or scaffold. These

scaffolds, too, can vary in terms of the material used, which in turn will bring unique physical properties that can alter the cell preparations function. This technique has demonstrated success in the preclinical arena as evidenced by a 2017 review of 11 preclinical studies. Here, using various animal models, the authors reported an average fusion rate for autograft of 59.8% across the studies versus 73.7% for stem cell-based grafts²⁰³. However, this success has not so readily translated to the clinical space. In recent years, a number of studies have directly compared outcomes of CBTs to autologous bone graft with varying results²⁰⁴⁻²⁰⁹. The goal of this systematic review and meta-analysis is to compare and contrast these studies in terms of safety, efficacy and long term outcomes, thus providing the current and relevant evidence for orthopaedic surgeons to make informed choices regarding this rapidly developing field.

7.3 Methods

A search was conducted by two authors (FN and AG) for articles published up to 1 March, 2023 using PubMed, EMBASE and the Cochrane Central Register of Controlled Trials. This study was registered on the PROSPERO database, an international prospective register of systematic reviews (<https://www.nihr.ac.uk/>). The electronic search results were limited to human studies, published in English. The following keywords were used for spine fusion (spine fusion OR spinal fusion OR spine arthrodesis OR spinal arthrodesis OR lumbar fusion OR lumbar arthrodesis) and for MSC and BMA (stem cell OR stem cells OR progenitor cell OR Osteoprogenitor cell OR bone marrow). Discussion with senior authors was conducted if disagreement ensued regarding study selection.

Studies were included if they met the following criteria: (i) randomised control trials (ii) written in English (iii) human studies (iv) comparative studies (v) used MSCs or BMA (vi) spinal fusion is the primary outcome measure. Exclusion criteria included: (i) non-randomised studies (ii) paediatric cases (iii) non-comparative studies or absence of control group.

For the included articles the following data were extracted: number of patients, type of grafts used, fusion assessment method, follow-up duration, fusion rates, clinical outcomes and complications. All relevant information was collected by two independent reviewers. Statistical analysis was performed using R (version 4.3.0). The I^2 statistic was

used to quantify heterogeneity between studies. Results are expressed as risk ratio for dichotomous outcomes and as mean for continuous outcomes, with a 95% confidence interval. Heterogeneity values were interpreted according to Cochrane values; (i) 0%-40% = low degree of heterogeneity (ii) 30%-60% = moderate degree of heterogeneity (iii) 50%-90% = substantial degree of heterogeneity (iv) 75%-90% = considerable degree of heterogeneity. A p-value of <0.05 was considered to be statistically significant.

The *Methodological Quality of Evidence (MQOE)* was assessed using the Risk Of Bias 2 (RoB-2) tool and Risk of Bias In Non-Randomised Studies (ROBINS) tool developed by Cochrane for evaluating bias in randomised and non-randomised studies.

7.4 Results

Our initial search returned 2,340 results. Following initial title and abstract screening, 50 articles were eligible for full text review. Finally, ten studies fulfilled the inclusion criteria for qualitative synthesis and quantitative analysis²⁰⁴⁻²¹³ (Figure 7-1).

Of the 10 studies included in this systematic review there were a total of 465 patients included. 273 patients received treatment with a CBT and 276 were included in the control group, receiving treatment with ICBG. The mean number of patients per study was 43.8 (std dev. 28.81, range 12 – 100). No studies included less than 10 patients. Studies typically included adult patients with degenerative spondylolisthesis and degenerative disc disease. Typical exclusion criteria within the primary studies included; positive HIV, hepatitis or syphilis serology, immunosuppression, spinal neoplasm, smoking, and significant haematological or chemistry lab abnormalities contraindicating surgery (Table 7-1) .

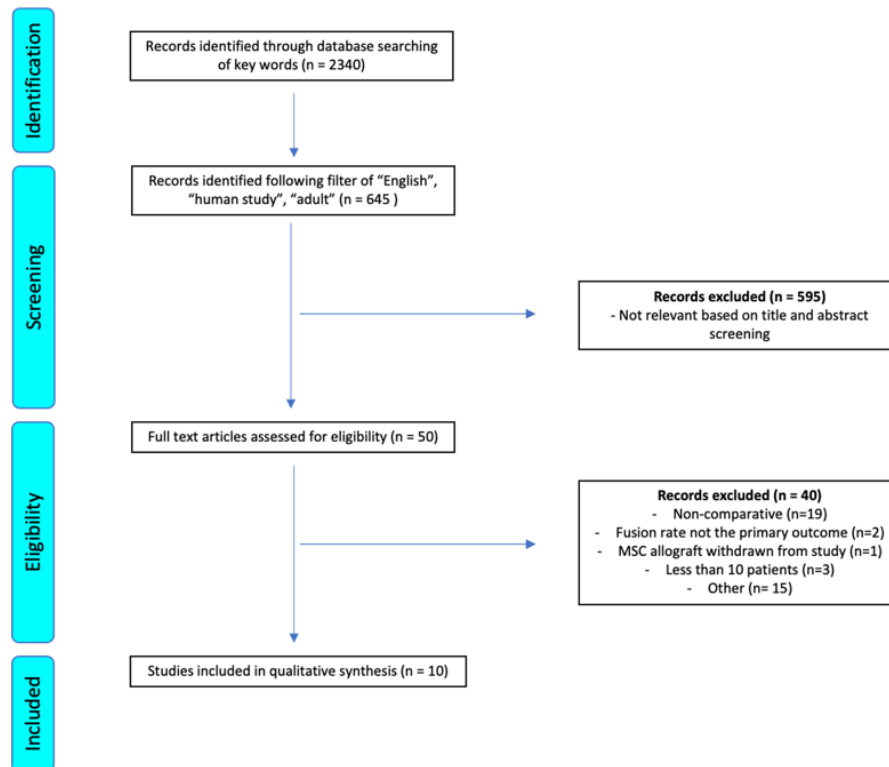


Figure 7-1 PRISMA flowchart of the literature search

7.4.1 Fusion Assessment

Two studies, Hart et al²⁰⁵ and Garcia et al²⁰⁶, demonstrated CBT to be significantly more successful in terms of fusion rates compared to ICBG. Garcia et al demonstrated a 76.5% fusion rate on the side treated with MSC and allograft at 12 months compared to 65.7% on the control side, using radiographic CT measurements. Hart et al²⁰⁵ demonstrated that in the BMA group, 80% had evidence of at least unilateral continuous bridging bone between neighbouring vertebrae at 24 months, compared to 40% in the control group. The remaining eight studies demonstrated equivocal results. No study found that CBT to be inferior. The radiographic assessment of specific cohorts is outlined below:

7.4.2 Radiographic Assessment of Degenerative Cohorts

Three studies²⁰⁴⁻²⁰⁶ reported on fusion rates for degenerative cohorts as evaluated on X-ray at final follow up. No difference was seen in fusion rates between the two groups (44/93, 47.3% vs 36/102, 35.2%; RR 1.55; p=0.51)Figure 7-2. Four studies²⁰⁵⁻²⁰⁸ reported on fusion rates for degenerative cohorts as evaluated on CT at final follow

up. No difference was seen in fusion rates between the two groups (94/120, 78.3% vs 74/121, 61.1%; RR: 1.22; p=0.34) (Figure 7-2).

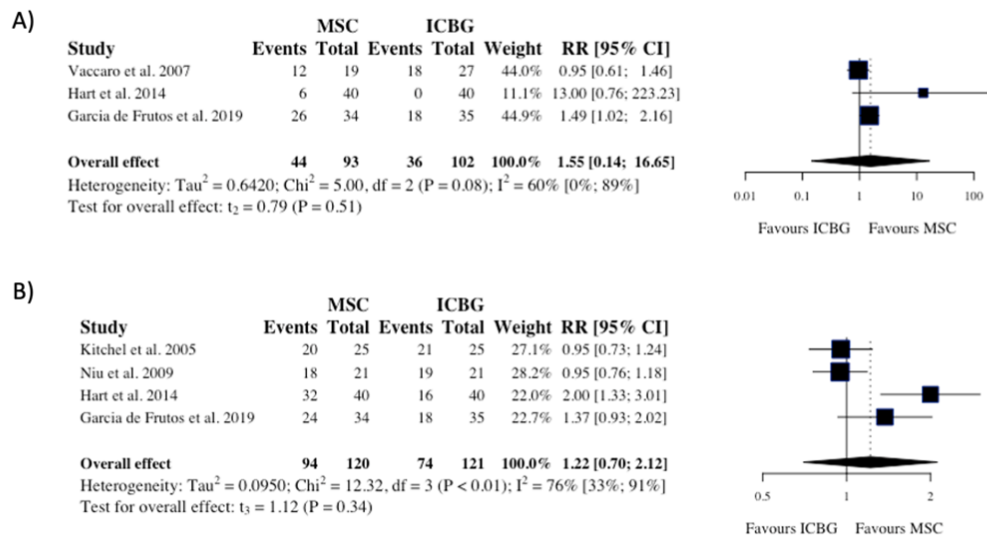


Figure 7-2 Forest plots detailing studies which examined degenerative cohorts; A) Degenerative Cohorts Fusion Rates as Evaluated on plain radiograph at Respective Final Follow-up B) Degenerative Cohorts Fusion Rates as Evaluated on CT at Respective Final Follow-up

7.4.3 Radiographic Assessment as Evaluated on Plain Radiographs

Three studies reported on fusion rates using plain radiographs at the 12 month time interval, with no difference seen between the groups (63/109, 57.7% vs 49/110, 44.5% ; RR: 1.55; p = 0.49) Figure 7-3. Three studies^{204,205,212} reported on fusion rates as evaluated on plain radiograph at 24 months follow up. No difference was seen in fusion rates between the two groups (68/109, 62.3% vs 69/117, 58.9%; RR : 1.27; p = 0.62). Eight studies^{205-211,213} reported on fusion rates as evaluated on plain radiograph at respective final follow up. No difference was seen in fusion rates between the two groups (169/207, 81.6% vs. 143/200, 71.5%; RR = 1.08; p = 0.53) (Figure 7-3).

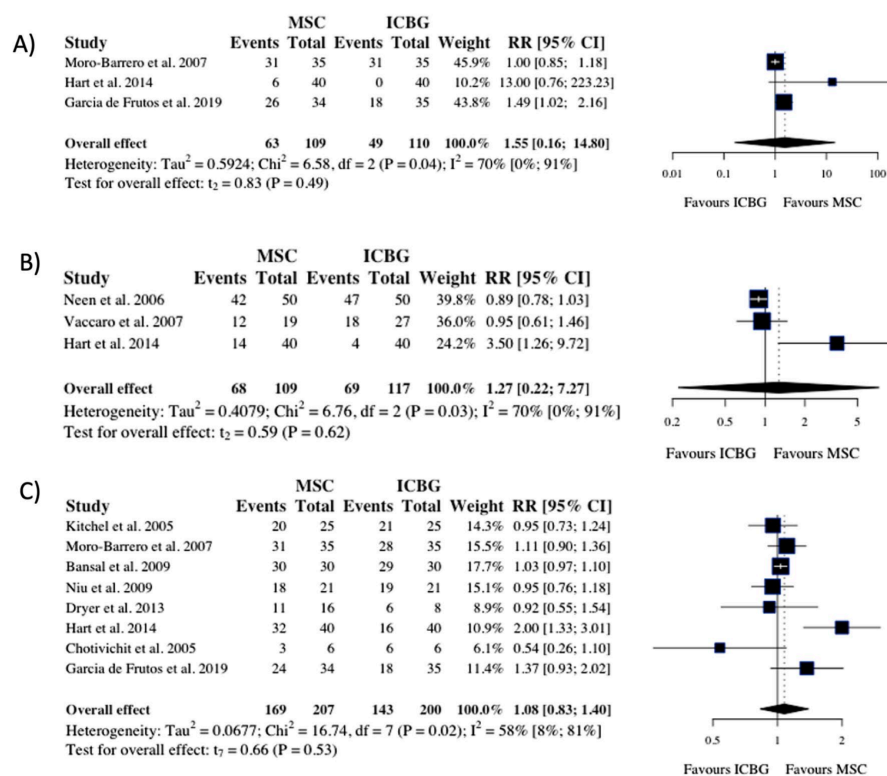


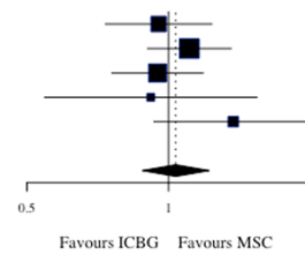
Figure 7-3 Forest plots of studies using plain radiographs to measure fusion success A) Fusion Rates as Evaluated on plain radiograph at 12 months B) Fusion Rates as Evaluated on plain radiograph at 24 months C) Fusion Rates as Evaluated on plain radiograph at Respective Final Follow-Up

7.4.4 Radiographic Assessment as Evaluated on Computed Tomography (CT)

Five studies²⁰⁶⁻²¹⁰ reported on fusion rates assessed by CT at 12 months. No difference was seen in fusion rates between the two groups (104/131, 79.4% vs 92/124, 74.2%; $\text{RR} = 1.04$, $p = 0.58$). Four studies^{205,207,208,213} reported on fusion rates assessed by CT at 24 months. No statistically significant difference was seen in fusion rates between the two groups (113/116, 97.4% vs 85/116, 73.3%; $\text{RR} = 1.27$; $p = 0.34$) (Figure 7-4).

A)

Study	MSC		ICBG		Weight	RR [95% CI]
	Events	Total	Events	Total		
Kitchel et al. 2005	20	25	21	25	21.3%	0.95 [0.73; 1.24]
Moro-Barrero et al. 2007	31	35	28	35	34.7%	1.11 [0.90; 1.36]
Niu et al. 2009	18	21	19	21	29.0%	0.95 [0.76; 1.18]
Dryer et al. 2013	11	16	6	8	5.4%	0.92 [0.55; 1.54]
Garcia de Frutos et al. 2019	24	34	18	35	9.6%	1.37 [0.93; 2.02]
Overall effect	104	131	92	124	100.0%	1.04 [0.88; 1.22]
Heterogeneity: $\tau^2 = 0$; $\chi^2 = 3.66$, $df = 4$ ($P = 0.45$); $I^2 = 0\%$ [0%; 77%]						
Test for overall effect: $t_4 = 0.60$ ($P = 0.58$)						



B)

Study	MSC		ICBG		Weight	RR [95% CI]
	Events	Total	Events	Total		
Kitchel et al. 2005	25	25	21	25	25.9%	1.19 [1.00; 1.40]
Bansal et al. 2009	30	30	29	30	26.9%	1.03 [0.97; 1.10]
Niu et al. 2009	18	21	19	21	25.1%	0.95 [0.76; 1.18]
Hart et al. 2014	40	40	16	40	22.1%	2.45 [1.69; 3.56]
Overall effect	113	116	85	116	100.0%	1.27 [0.65; 2.46]
Heterogeneity: $\tau^2 = 0.1602$; $\chi^2 = 22.84$, $df = 3$ ($P < 0.01$); $I^2 = 87\%$ [68%; 95%]						
Test for overall effect: $t_3 = 1.14$ ($P = 0.34$)						

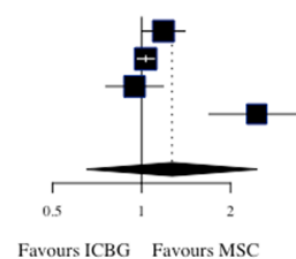


Figure 7-4 Forest plots of studies using computed tomography (CT) to evaluate fusion rates A) Fusion Rates as Evaluated on CT at 12 months B) Fusion Rates as Evaluated on CT at 24 months

7.4.5 Cell Preparation Methods

The methods of obtaining cells and type of scaffolds used varied between the studies. In Garcia de Frutos et al²⁰⁶ and Hart et al²⁰⁵ bone marrow is mixed with allogenic bone. In both Kitchel²⁰⁷ and Neen et al²¹², a collagen substitute is used in lieu of bone and the collagen substitute is then mixed with BMA to comprise the cell-based graft. In Neen et al²¹², a bone graft substitute called Healos is used in combination with bone marrow. Healos is a type 1 collagen / hydroxyapatite matrix which when soaked for 20 minutes in BMA becomes osteoinductive. In Moro-Barrero et al²⁰⁹ and Bansal et al²¹³, calcium phosphate solutions are mixed with bone marrow aspirate to form the graft. Vaccaro et al²⁰⁴ used a demineralized bone matrix putty enriched with aspirated bone marrow.

7.4.6 Functional Outcomes

Six studies examined clinical outcomes as a secondary measure. Among these, four studies employed formal scoring methods such as VAS and/or ODI scores, while two studies relied on patient-reported pain (Table 7-2). When comparing autologous bone graft and cell-based therapies, multiple studies assessed both clinical outcomes and fusion rates. VAS scoring was utilized in three studies^{206,207,210}, while ODI scoring was employed in four studies^{206,207,210,211}. Dryer et al²¹⁰ did not include raw data but observed a significant and comparable improvement in ODI scores and a reduction in VAS scores across both study groups.

In Kitchel et al²⁰⁷, patients received both CBT and ICBG on each side of the fusion construct and therefore, with internal controls, they could not be compared individually. In Garcia et al.²⁰⁶, the mean VAS baseline scores for both MSC and ICBG groups indicated moderate to severe lumbar (6.7 [5.8–7.5] vs. 6.8 [6.0–7.6], $p=.859$) and sciatic pain (7.0 [6.1–8.0] vs. 7.3 [6.6–8.1], $p=.916$). Both groups demonstrated a significant improvement in lumbar and sciatic pain scores overall ($p<.0001$ for both groups at all time points), but there were no significant differences between the groups at any time point. Garcia et al²⁰⁶ also analysed ODI scores. The mean baseline scores for MSC (40.6 [35.7–45.6]) and ICBG (43.4 [37.7–49.1]) groups were comparable, indicating moderate to severe disability ($p=.4563$). No significant differences in mean ODI scores were found between the groups at any time point when compared to baseline scores. However, both the MSC and ICBG groups exhibited significant decreases in mean ODI scores at 3, 6, and 12 months, respectively. In Chotivichit et al²¹¹, ODI scores were utilized to assess clinical outcomes. Both MSC and ICBG groups showed postoperative decreases in ODI scores, but no significant differences in clinical outcomes were observed between the groups at the 3- and 6-month time points.

In all four studies, both treatment groups experienced a significant reduction in pain measured by VAS and an improvement in function assessed by ODI. Additionally, these studies consistently indicated that there were no notable differences in clinical outcomes between the groups receiving autologous bone graft and those receiving cell-based therapy.

7.4.7 Complications

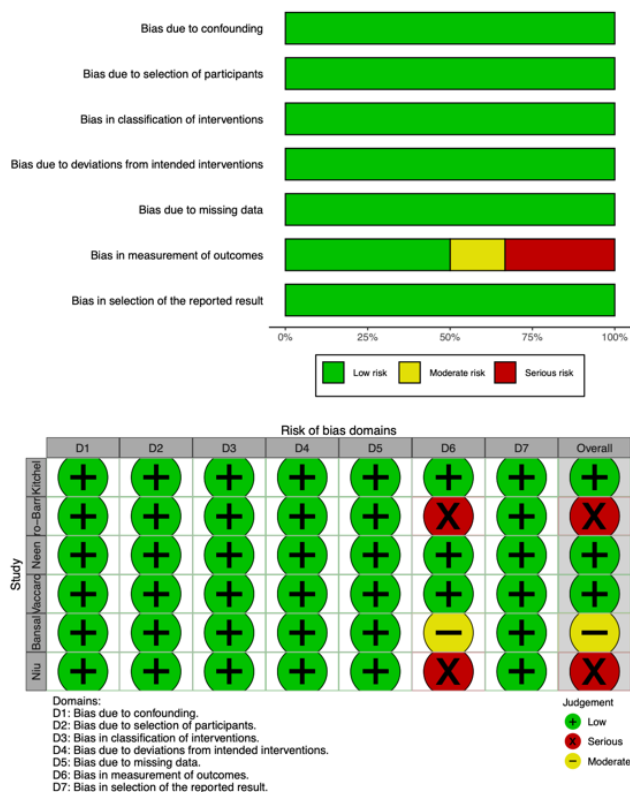
The included papers in this review also addressed postoperative complications. Notably, four studies, Garcia de Frutos et al²⁰⁶, Kitchel²⁰⁷, Neen et al²¹² and Bansal et

al²¹³, reported chronic donor site pain after ICBG in up to 28% of patients. No similar complications were noted following BMA for CBT. Garcia de Frutos et al²⁰⁶ reported back pain to be the most common complaint post-operatively with 54.3% of ICBG patients experiencing back pain compared to 35.3% of MSC patients. Hart et al²⁰⁵ reported no difference in mean length of hospital stay between groups. There were no complications specific to BMA reported.

7.4.8 Risk of Bias Assessment

The risk of bias assessment was performed using the ROBINS-I tool for the non-randomised, and the ROB-2 tool for the randomised studies in this review. Overall, there was a low degree of bias in the cohort as a whole. However, there were some concerns of bias, particularly in the reporting of outcome data domains in 4 of the included studies^{208,209,211,213}, as seen in Figure 7-5.

A)



B)

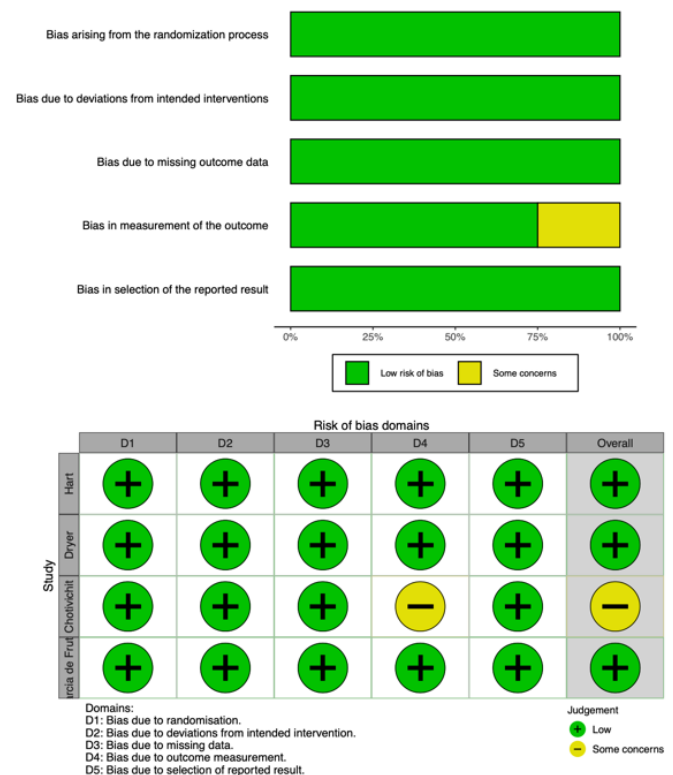


Figure 7-5 Graphical representation of risk of bias assessments A) Risk of Bias in Non-Randomised Studies (ROBINS) tool and B) the Risk Of Bias 2 (RoB-2) tool

7.5 Discussion

This systematic review is the first meta-analysis exploring the use of CBT grafts in spinal fusion surgery. We hope that this study will inform and educate clinical decision makers of the best available evidence in this field. Here, 10 studies were chosen from a comprehensive literature search. These studies represent a high level of evidence with 4 randomised controlled trials and 6 prospective comparative studies included. Overall, we report a favourable non-inferiority of MSC based scaffolds to ICBG.

In recent years, CBT is gaining favour as an alternative to ICBG. Numerous preclinical studies have shown comparable outcomes using MSCs and progenitor cells for spinal fusion.¹⁷ The results of our systematic review report mixed, but positive outcomes for the use of cell based grafts. Radiographically, two studies, Garcia et al and Hart et al, described a statistically significant improvement in spine fusion rates using CBTs compared to ICBG. The other eight studies in this review found the two methods equivocal. Ultimately though, CBT shows non-inferiority to ICBG.

Spinal fusion surgery can carry significant morbidity in the form of infection, neurovascular damage and, most commonly, pain. Postoperative pain at the site of graft harvest is the most commonly reported complication and has been reported in 2.8% to 27.9% of those undergoing spinal fusion with ICBG¹³. Therefore, the desire to avoid harvesting bone graft is desirable. Other complications associated with posterior iliac crest graft harvest include haematoma (9.5%) and seroma (14.3%) formation and superficial infection (9.5%).¹⁴ A study from 2009 reported that at follow up 3.5 years post lumbar spinal fusion surgery, 24% of patients reported harvest site numbness and 13% reported that the numbness was bothersome¹⁵. However, more recent literature is conflicting on the morbidity related to more modern bone graft harvesting techniques. Banwart et al²¹⁴ stated that severe and major complications can be avoided provided certain procedural refinements, such as limiting subcutaneous dissection and tension free skin closure, are employed. Moreover, continuous technical refinements have been proposed in modern literature aiming to reduce the harmful sequelae of this procedure²¹⁵⁻²¹⁷. Chronic donor site pain is the most commonly reported adverse event associated with ICBG and was a complication described throughout all the studies included in this review for patients undergoing ICBG. One study found the number of patients experiencing pain at the graft site two years post-operatively was as high as 28%. The morbidity associated

with BMA is considered significantly less than that associated with bone graft harvesting¹⁹. However, a recent randomised trial found that patients undergoing ICBG using the lumbar spinal incision approach could not reliably state which iliac crest had been harvested, nor did the donor site have increased pain compared to the contralateral untouched side²¹⁸. These findings have been echoed in other more recent studies²¹⁹⁻²²¹. Therefore, the overall morbidity of modern ICBG may be reduced compared to the traditional complication rates quoted. Our study demonstrated no observed complication rates pertaining to the BMA, in contrast to the increased pain among those undergoing bone graft harvest.

One inconsistency noted in this review, which is readily apparent, is the varying techniques with which the cell sample is collected, prepared and delivered. This degree of variation, while necessary to optimise the procedure in the preclinical space, is at present a cause of controversy and limiting the progression of these technologies to clinical practice. The methods included in our review ranged from applying a fresh bone marrow aspirate, concentrated bone marrow aspirates or culture expanded MSC cells to a number of different synthetic bone grafts or allogenic bone grafts. One of the root cause of this heterogeneity can be traced to the lack of a precise definition of MSCs and the current controversy surrounding the use of the term “stem cells”³⁰. This leads to both inconsistent and inadequate reporting of cellular preparations and there have been numerous calls to establish a unifying definition of MSCs with stricter regulatory requirements.

While beyond the scope of this study, there are a number of other products or avenues being explored to replace the use of ICBG. A number of demineralised bone matrix (DBM) products are in use in spinal surgery, namely; Accell Connexus (Citagenix, Laval, Canada), Allomatrix, Grafton DBM, and Osteofil (Medtronic, Minneapolis, Minnesota). These products have shown efficacy as bone graft extenders, when compared to bone graft alone²²² and theoretically could be used as carriers or scaffolds in conjunction with cell-based techniques. Synthetic bone grafts too serve as osteoconductive compounds capable of providing a structural lattice for bone growth. Beta tricalcium phosphate is one such material, which was used in some of the studies in this review, and has been used as a stand-alone product (Vitoss, by Orthovita) in posterolateral spinal fusion. Osteoinductive products incorporating small molecules rose to the fore in the late 1990's, namely the bone morphogenetic proteins (BMP) 2 and 7.

While controversy exists regarding the validity of the original clinical trials supporting their use, BMP-2 products are still used in orthopaedic spinal surgery and have been extensively reviewed elsewhere²²³. i-Factor Bone Graft, by Cerapadics, uses a bovine-derived hydroxyapatite matrix combined with a synthetic 15 amino acid residue (ABM/P-15) which has shown good success in maxillofacial surgery²²⁴. At 2 year follow-up, in a RCT, ABM/P-15 demonstrated noninferiority to local bone autograft in anterior cervical discectomy and fusion (ACDF)²²⁵. Jacobsen et al²²⁴, in a RCT, demonstrated superior radiographic but equivalent clinical outcomes when comparing ABM/P-15 to a bone allograft in non-instrumented lumbar fusion.

This study has a number of limitations. Firstly, we included only studies published in the English language. Like any systematic review or meta-analysis, we are limited by the quality of included studies. We attempted to mitigate this factor by including the highest available evidence on this topic, which includes randomised controlled trials and prospective cohort studies with control groups. However, these studies in turn are limited by two year-follow up. Only four studies measured patient reported outcomes (PROMs) using the VAS and ODI scores, as discussed in the results section. PROMs, which aim to measure clinically important outcomes to the patient, have become increasingly important in clinical research and their exclusion limits the ability to measure the potential benefit of an intervention to patients²²⁶. As discussed above, the variation of cell preparations across the review is a considerable source of heterogeneity and is therefore a limitation.

Nevertheless, the future outlook of this space is very exciting. As our understanding of cell based bone biology continues to grow, so too are the potential therapeutic strategies that will come into use²²⁷. A balance will need to be sought between the standardisation of techniques in clinical use and the continued evolution of these products.

7.6 Conclusion

Cell-based therapy offers a promising alternative to ICBG in spinal fusion surgery, which could help reduce the associated morbidity to patients. This review found that cell based therapy is non-inferior to iliac crest bone graft and may offer patients an alternative treatment option with fewer complications and reduced post-operative pain. However, the literature to date is limited by heterogeneity of the cell preparation and

grafting process. Future research with a unified approach to the cell preparation process is required to fully delineate the potential advantages of this technology.

Table 7-1 Characteristics of the included studies

Study	Design	Patient Numbers	Indication for fusion surgery	Radiographic Evaluation	Level of Evidence	Age Range	Gender	Outcome (stem cells vs ICBG)
Hart et al ²⁰⁵ 2014	RCT	80	Degenerative disc disease and spondylolisthesis	Plain radiograph and CT	I	42 - 80	Male: 22 (27.5%) Female: 58 (72.5%)	Favoured stem cells
Dryer et al ²¹⁰ 2013	RCT	24	Non-specified	Plain radiograph and CT	I			Equivocal
Chotivichit et al ²¹¹ 2016	RCT	12	Spondylolisthesis	3D-CT imaging	I	42 - 72	Male: 5 (41.66%) Female: 7 (58.3%)	Equivocal
Garcia et al ²⁰⁶ 2019	Multicentre RCT (phase I-II clinical trial)	65	Degenerative disease and spondylolisthesis	Plain radiograph and CT	I	28-85	Male: 9 (26.47%) Female: 25 (73.53%)	Favoured stem cells
Kitchel ²⁰⁷ 2006	Prospective Cohort	25	Degenerative disease	CT	II	36-72	Male: 11 (44%) Female: 14 (56%)	Equivocal
Moro-Barrero et al ²⁰⁹ 2007	Prospective Cohort	35	Non-specified	Plain radiograph	III	Average age 59.2	Male: 24 (68.57%)	Equivocal

							Female: 11 (31.43%)	
Neen et al ²¹² 2006	Prospective Case Control	100	Non-specified	Plain radiograph	III	Median age 49	Male : 50 (50%) Female: 50 (50%)	Equivocal
Niu et al ²⁰⁸ 2009	Randomized Trial	21	Degenerative disease	CT	II	27-75	Male: 7 (33.33%) Female: 14 (66.66%)	Equivocal
Vaccaro et al ²⁰⁴ 2007	Prospective cohort	73	Degenerative disease	Plain radiographs	III	Average age 54.6	Male: 23 (50%) Female: 23 (50%)	Equivocal
Bansal et al ²¹³ 2009	Prospective cohort	30	Trauma	CT	III			Equivocal

Table 7-2 describing the comparative methodology of the included studies.

Study	Control Group	Stem Cells Sample	Surgical Methods	Radiographic follow up	Clinical Outcomes
Hart et al ²⁰⁵ 2014	Spongious allograft bone chips	Bone Marrow Concentrate with	Lumbar or lumbosacral	Radiograph at 12 and 24 months. CT at 24 months.	None

		allograft spongiuous bone chips	posterolateral fusion (PLF)		
Dryer et al²¹⁰ 2013	ICBG	Immunoselected and culture-expanded allogeneic mesenchymal precursor cells combined with a carrier (hydroxyapatite, β -tricalcium phosphate, and collagen)	posterior lumbar interbody fusion with instrumentation and interbody PEEK cages	Radiograph at 3, 6, 9, 12 and 24 months. CT at 3, 6, 12 and 24 months.	VAS and ODI scores
Chotivichit et al²¹¹ 2016	Local bone graft - autologous bone chips harvested during laminectomy	Bone Marrow Concentrate with autologous bone graft	PLF after decompressive laminectomy and pedicle screw instrumentation	3D-CT at 1 week and 6 months	ODI score
Garcia et al²²⁸ 2020	ICBG	MSCs harvested from bone marrow 21 days preoperatively cultured onto allogenic cancellous bone cubes	Transforaminal lumbar interbody fusion	Radiograph at 3, 6 and 12 months. CT at 6 and 12 months.	VAS and ODI scores
Kitchel et al²⁰⁷ 2005	ICBG	Bone marrow aspirate with a mineralised collagen bone graft	Posterior lumbar interbody fusion	CT at 12 and 24 months	VAS and ODI scores
Moro-Barrero et al²⁰⁹ 2007	ICBG	Bone Marrow Aspirate with biphasic calcium	Posterolateral fusion - stem cells on the right side	Radiograph at 3, 6, 12 and 24 months. CT at 24 months.	None

		phosphate (BCP) graft	of the spine and ICBG on the left side of the spine.		
Neen et al²¹² 2006	ICBG	Bone marrow aspirate applied to Healos strips	Patients underwent either posterolateral fusion, posterior lumbar interbody fusion or 360° front and back fusion	Radiograph at 24 months	Low back outcome scores and patient satisfaction Scores
Niu et al²⁰⁸ 2009	ICBG	Bone marrow aspirate mixed with autologous laminectomy bone chips	Posterolateral fusion - stem cells on one side of the spine and ICBG on the other side of the spine.	Radiograph at 3, 6, 9 and 12 months. CT at 12 months.	None
Vaccaro et al²⁰⁴ 2007	ICBG	Demineralised bone matrix putty enriched with aspirated bone marrow	Posterolateral lumbosacral spinal fusion	Radiograph at 24 months.	Low Back Pain Outcomes Assessment and patient reported severity of hip pain at the donor site and contralateral hip
Bansal et al²¹³ 2009	ICBG	Bone marrow aspirate mixed with hydroxyapatite and beta-tricalcium phosphate	Posterior fusion with stem cells on one side of the spine and ICBG on the other side of the spine.	Radiograph at 3, 6 and 12. CT at 12 months.	None

Chapter 8 Recombinant Human Bone Morphogenetic Proteins versus Iliac Crest Bone Graft in Spinal Arthrodesis: A Systematic Review of Overlapping Meta-Analyses

8.1 Abstract

8.1.1 Introduction

Recombinant bone morphogenetic proteins (rhBMP) are powerful alternatives to traditional bone graft for spinal fusion. Multiple randomised controlled trials and meta-analyses have been performed to establish their utility compared to the gold standard of autologous iliac crest bone graft (ICBG). Despite these studies, controversy remains over the ability of these products to achieve a successful bony fusion and the potential complications associated with their use. This is in part due to discordant results and conclusions drawn from the multiple meta-analyses performed on this subject. The aim of this study is to systematically review the published meta-analyses comparing rhBMP use and iliac crest bone graft in order to establish the best available evidence.

8.1.2 Methods

A systematic review of overlapping meta-analyses was performed according to the *Preferred Reporting Items for Systematic Reviews and Meta-Analysis* (PRISMA) guidelines. Methodological quality was assessed using the Oxford Levels of Evidence and the *Assessment of Multiple Systematic Reviews* (AMSTAR) instrument. The Jadad decision algorithm was used to establish the best available evidence.

8.1.3 Results

13 meta-analyses were included: 6 investigating rhBMP-2, 2 investigating rhBMP-7 and 5 investigating both rhBMP-2 and rhBMP-7. The mean AMSTAR grade was 7.385. Across the 13 included meta-analyses, 42 individual primary studies were included. The mean number of primary studies a meta-analysis included was 3.282. The Jadad decision algorithm identified 2 studies as the overall best available evidence. These studies reported in favour of rhBMP-2 compared to ICBG (RR 1.12 at 12 months) for successful fusion.

8.1.4 Conclusions

This systematic review demonstrated inconsistent results among the included meta-analyses. Based on the best available evidence, the use of rhBMP-2 results in improved fusion rates compared to ICBG. There appears to be a trend towards a greater adverse risk event profile when using rhBMP-2. However, it remains difficult to ascertain the significance of this trend. These potential risks should be weighed against the potential morbidity associated with the ICBG procedure.

8.2 Introduction

Spinal fusion (arthrodesis) was first proposed as a technique to surgically stabilise the spine in 1911 by Hibbs and Albee, using autologous bone graft for posterior lumbar fusion²²⁹. Autologous bone graft has remained a critical component to promote spinal fusion, with iliac crest bone graft (ICBG) providing the gold standard of donor tissue. ICBG harvesting is a procedure with associated with morbidity rates as high as 30%. Chronic pain at the donor site is often cited as a common complication, with a reported incidence ranging from 25 – 60%^{214,230,231}. In addition, there is a limit to the total quantity of the autologous graft material that may be harvested. Notably, the popularity of autogenous bone grafting has grown in recent decades, potentially reflected by a simultaneous increase in the number of spinal fusion procedures performed over the same period. Ludwig et al reported that over 200,000 autogenous bone grafting procedures were performed annually by 2000⁵. Therefore, it is not surprising that efforts have been made to seek alternative fusion promoters to the traditional bone graft, to negate aforementioned limitations and complications associated with ICBG.

Recombinant human bone morphogenetic proteins (rhBMP), intracellular osteoinductive signalling factors that belong to the transforming growth factor- β superfamily of signalling proteins, represent an alternative material to ICBG. Thus far, two rhBMP proteins are used in licensed products which have been widely used to augment bone healing in spinal fusion surgery in lieu of ICBG. rhBMP-2 is a constituent used in the INFUSE bone graft (Medtronic, Memphis, TN, USA). It obtained FDA approval in 2002 for single level anterior lumbar interbody fusions (ALIF) in skeletally mature individuals with single-level degenerative disk disease between L4 and S1. OP-1 Putty (Osteogenic Protein -1, incorporating rhBMP-7) (Stryker, Michigan, USA) obtained regulatory approval by the FDA for use in compromised patients undergoing revision posterolateral fusion in whom autologous bone graft harvest was not feasible or not expected to promote fusion.

Since the advent of the rhBMPs, several randomised clinical trials (RCTs) have been conducted to compare their performance with ICBG. However, their findings are inconsistent in terms of both the benefits of fusion outcomes and the potential complications associated with the use of rhBMPs. As a result of this, a number of meta-analyses of RCTs have been performed to compare these graft materials in lumbar spinal fusion. However, these overlapping meta-analyses have demonstrated inconsistent,

discordant conclusions and therefore, uncertainty remains for surgeons deciding whether to ultimately use these products versus autologous bone graft.

In recent years, systematic reviews of overlapping meta-analyses have been used across the medical literature to select the highest quality level of evidence by evaluating meta-analyses with discordant results on a particular topic²³²⁻²³⁴. To our knowledge, there is at present no systematic review of overlapping meta-analyses investigating the use of the rhBMP proteins in spinal fusion compared with ICBG. Therefore, the objective of this review was to perform a systematic review of overlapping meta-analyses examining the use of both rhBMP-2 and rhBMP-7 versus ICBG in lumbar spinal fusion and to provide treatment recommendations based on the best available evidence. This will assist clinicians in selection among conflicting study results.

8.3 Methods

This study was conducted according to the *Preferred Reporting Items for Systematic Reviews and Meta-analysis* (PRISMA) statement²³⁵. The design of this study was influenced from similar publications that exist in the literature and concern other topics of interest^{232,234} (Figure 8-1).

8.3.1 Literature Search

On the 1st January 2022, the PubMed, Embase, and Cochrane were systematically searched. Search terms included; “bone morphogenetic protein” AND “meta-analysis”, “BMP” AND “meta-analysis”, “lumbar fusion” AND “meta-analysis”, “spinal fusion” AND “meta-analysis”, and “bone morphogenetic protein” “BMP” “spinal fusion” meta-analysis”. The search was independently performed by two reviewers (D.P.A and J.M.M). Articles were screened by title and abstract initially, followed by full text review. Furthermore, the reference section of included articles were scanned for further relevant articles.

8.3.2 Eligibility Criteria

The inclusion criteria were as follows: 1) articles comparing the use of rhBMP to bone graft in lumbar spinal fusion 2) meta-analyses 3) at least one outcome (e.g. functional scores and complications) 4) articles examining rhBMP-2, rhBMP-7 and both rhBMP-2 and -7 were included. Exclusion criteria included: 1) articles not in the English language 2) non-review articles pertaining to primary studies (RCT/prospective/retrospective) and 3) review articles with no advanced statistics (meta-analysis) performed 4) articles concerning spinal regions other than the lumbar spine.

8.3.3 Data Extraction

Two authors (D.P.A and J.M.M) independently extracted the following data from the included studies: first author, year of publication, database for search, primary study design, number of RCTs included, heterogeneity or subgroup analysis, and meta-analysis results.

8.3.4 Quality Assessment

The methodological quality was evaluated by the *Oxford Levels of Evidence* and the *Assessment of Multiple Systematic Reviews* (AMSTAR) instrument²³⁶. The AMSTAR

score ranges from 0-11 with a higher score indicating a higher quality review. In general, a score of 8 – 11 indicates a good quality study, 4 – 7 a moderate quality review and 1 – 3 of low quality. The Jadad decision algorithm is a tool used to provide treatment recommendations among meta-analyses with discordant results and was used to investigate the source of inconsistency among the included studies. This tool examines differences in clinical question, inclusion and exclusion criteria, data extraction, quality assessment, data pooling and statistical analysis²³⁷.

8.4 Results

8.4.1 Literature Review

The initial database search yielded 771 potential articles for review. Following screening of title and abstracts, 755 articles were excluded due to non-relevance. During full-text review, 1 article was excluded for dealing exclusively in the setting of tumour surgery, 2 articles were excluded for examining the rhBMP in the setting of cervical surgery only, 1 network meta-analysis examining multiple bone graft substitutes was excluded²³⁸, 1 meta-analysis whose aim was to determine optimal dosing of rhBMP in interbody fusion was excluded²³⁹. This yielded 13 articles for inclusion (Table 8-1)^{223,240-251}. Of these 13 articles, 6 papers included rhBMP-2 only^{223,245-249}, 2 papers included rhBMP-7 only^{250,251}, and 5 papers included both rhBMP-2 and rhBMP-7²⁴⁰⁻²⁴⁴ (Table 8-2). No significant difference was noted in the publication years between the three groups ($P = 0.805$) (Figure 8-2).

Table 8-1 The characteristics of the included meta-analyses

First Author	Publication Year	Journal	Impact Factor	Date of last literature search	Number of included RCTs	Number of included studies
Papakostidis	2008	Spine	2.903	Not listed	6	7
Chen	2012	Archives of Orthopaedic and Trauma Surgery	2.07	Feb-12	10	10
Simmonds	2013	Annals of Internal Medicine	21.317	Jun-12	12	54
Fu	2013	Annals of Internal Medicine	21.317	Dec-11	13	125
Noshchenko	2014	Journal of Spine Disorders and Techniques	1.401	May-12	8	8
Zhang	2014	Plos One	2.74	Nov-13	19	19
Vavken	2015	Global Spine Journal	2.18	Nov-14	6	6
Laurie	2016	Spine	2.903	Not listed	10	10
Ye	2017	Journal of Bone and Mineral Metabolism	2.294	Jul-16	5	5
Poorman	2017	European Spine Journal	2.458	Aug-15	2	4
Mariscal	2019	Journal of Bone and Mineral Metabolism	2.294	Jul-18	6	6
Han	2019	Chinese Journal of Traumatology	1.07	2018	7	11
Liu	2020	Spine	2.903	May-19	20	20

Table 8-2 Types of rhBMP investigated in included meta-analyses and principle conclusions drawn. Traffic light indication of conclusion recommendations towards the use of rhBMP

Author (Year)	BMP-2 only	BMP-7 only	BMP-2 and BMP-7	Conclusions	For / Against BMP
Papakostidis (2008)			X	"the radiographic results appeared better in the group of BMPs, the exact role of type, dose and carrier of BMPs... need further clinical delineation."	
Chen (2012)	X			"RhBMP-2 was superior to the ICBG for achieving fusion success and avoiding reoperation. However... Original, industry-sponsored trials underestimated rhBMP-2-related adverse events. There are still security risks in the use of rhBMP-2"	
Simmonds (2013)	X			"At 24 months, rhBMP-2 increases fusion rates, reduces pain by a clinically significant amount and increases early postsurgical pain compared with ICBG. Evidence of increased cancer incidence is inconclusive."	
Fu (2013)	X			"In spinal fusion, rhBMP-2 has no proven clinical advantage over bone graft and may be associated with important harms, making it difficult to identify clear indications for rhBMP-2."	
Noshchenko (2014)			X	"BMP, in particular rhBMP-2, is a good alternative to autogenous bone graft... However, the current study did not reveal evidence robust enough to develop strong medical recommendations concerning BMP use for lumbar arthrodesis in degenerative disk disease"	
Zhang (2014)			X	"BMPs in lumbar fusion can increase the fusion rate, while reduce the reoperation rate and operating time. However, it doesn't increase the complication rate, the amount of blood loss and hospital stay. No significant difference was found in the overall success of clinical outcome of the two groups."	
Vavken (2015)		X		"We were not able to find data in support of the use of rhBMP-7 for spine fusion. We found no evidence for increased complication or revision rates with rhBMP-7."	
Laurie (2016)	X			"Effects of rhBMP-2 may vary according to patient characteristics. Future studies should include planned subgroup analysis... in order to better identify those most likely to benefit."	
Ye (2017)		X		"the use of rhBMP-7 instead of ICBG produce no additional beneficial effect on the fusion rates, clinical success of ODI, overall adverse events, revision rates and duration of hospitalization in single level PLF. On the contrary, it appeared to yield lower fusion rate in the instrumented posterolateral fusion rates and cannot be recommended..."	
Poorman (2017)	X			"current literature shows BMP to be a safe and effective grafting technique in the treatment of ASD...the benefit of increasing the rate of fusion must be weighed against the increased risk of radiculitis and neurologic complications".	
Mariscal (2019)	X			"the use of BMP-2 in PLF reduced the surgical morbidity and had more beneficial effects on the fusion rate"	
Han (2019)			X	"current literature shows rhBMP is a safe and effective grafting material in the treatment of lumbar spondylolisthesis"	
Liu (2020)			X	"In lumbar fusion, rhBMP-2 exhibited a higher fusion success rate and reduced the risk of re-operation. No difference in complication rate is between rhBMP (rhBMP-2 and rhBMP-7) and ICBG. We suggest rhBMP especially rhBMP-2 as an effective substitute for ICBG for lumbar fusion."	

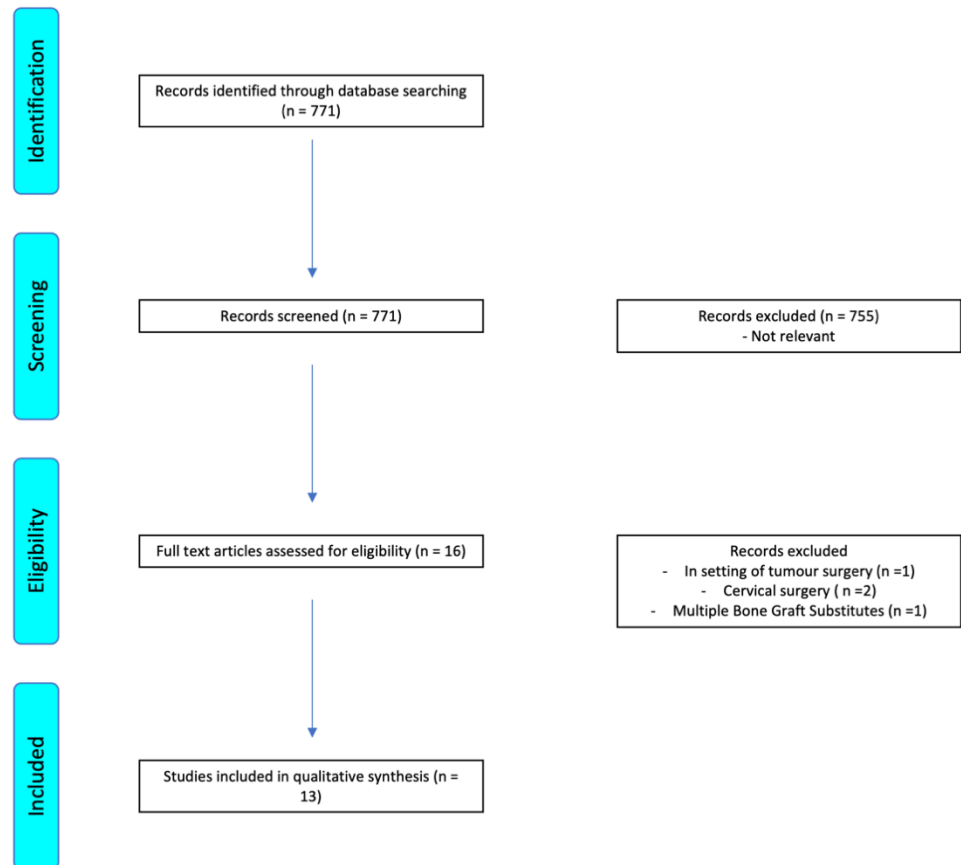


Figure 8-1 PRISMA flowchart of the performed literature search

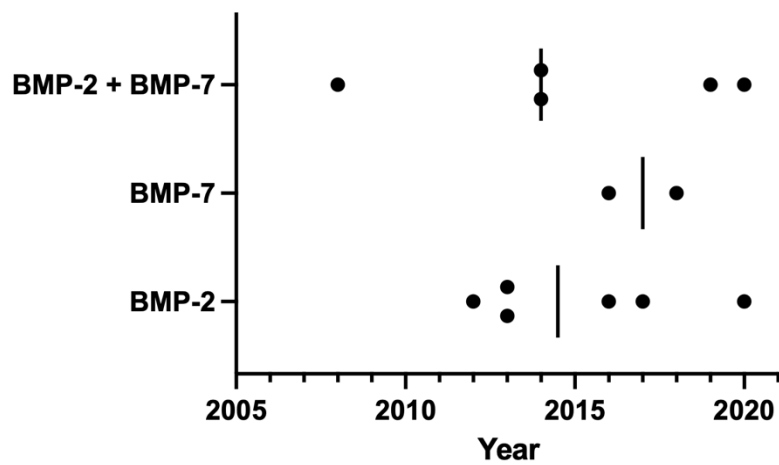


Figure 8-2 Publication years versus the type of BMP investigated in each meta-analysis, $P = 0.805$

8.4.2 Search Methodology

8 of the included studies restricted the publication language to the English language and only 1 study explicitly restricted search results based off publication status. Inconsistency was seen among the electronic databases used to perform the literature search. The Cochrane library (11 studies), followed by the PubMed and Embase databases (10 studies) were the two most common resources employed to conduct search strategies. Other commonly referenced databases included Medline (4 studies) and clinicaltrials.gov (3 studies). 9 of the studies included at least 1 other miscellaneous database in their search strategy. The search methodology findings are displayed in (Table 8-3).

8.4.3 Methodological Quality

8 studies^{240,241,244-247,250,251} included RCTs only, with the remainder incorporating both prospective^{223,242,248,249} and retrospective²⁴³ cohort studies in their analysis (Table 8-4). 4 studies incorporated the GRADE approach and 9 studies performed a sensitivity analysis. The mean AMSTAR grade was 7.385 (SD 1.981, range 4 – 11). 7 studies achieved an AMSTAR score of 8 or higher, indicating good methodological quality (Table 8-5). All included studies performed subgroup analysis. However, the parameters analysed varied between studies, as detailed in Table 8-6. Across the 13 included meta-analyses, 42 individual primary studies were included (Table 8-7). The mean number of primary studies a meta-analysis included was 3.282 (STD 2.339, range 1 – 9).

8.4.4 Results of Jadad decision algorithm

The Jadad decision algorithm was applied to determine which of the 13 included meta-analyses provided the best evidence for clinical decision making²³⁷. Given that 1) the included meta-analyses did not address the same study question 2) the reviews did not include the same primary trials and 3) the reviews did not have the same selection criteria, the Jadad decision algorithm recommends that the highest quality reviews be selected based on publication characteristics, methodology of the primary trials, language restrictions and whether an analysis of data on individual patients was included in the study. This yielded 2 high quality meta-analyses of individual patient data (IPD)^{223,249}, both utilising the same IPD dataset associated with the Yale University Open Data Access (YODA) project. However, these studies determined discordant conclusions. Simmonds et al²⁴⁹ concludes an overall positive finding that *“at 24 months, rhBMP-2 increases fusion rates, reduces pain by a clinical significant amount, and increases early*

postsurgical pain compared with ICBG” compared to the more negative finding by Fu et al²²³ that “in spinal fusion, rhBMP-2 has no proven clinical advantage over bone graft and may be associated with important harms”.

Table 8-3 Primary studies included in the meta-analyses.

Author (Year)	Restriction of publication language	Restriction of publication status	PubMed	Medline	Embase	Cochrane Library	Clinicaltrials.gov	Other
Papakostidis (2008)	Yes	No	Yes	Yes	-	Yes	-	No
Chen (2012)	Yes	No	Yes	-	Yes	Yes	-	No
Simmonds (2013)	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Fu (2013)	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Noshchenko (2014)	No	No	-	-	Yes	Yes	-	Yes
Zhang (2014)	No	No	Yes	Yes	-	Yes	Yes	Yes
Vavken (2015)	Yes	No	Yes	-	Yes	Yes	-	Yes
Laurie (2016)	Yes	No	-	-	-	-	-	No
Ye (2017)	Yes	No	Yes	-	Yes	Yes	-	Yes
Poorman (2017)	Yes	No	Yes	-	Yes	-	-	Yes
Mariscal (2019)	Yes	No	Yes	-	Yes	Yes	-	Yes
Han (2019)	No	No	Yes	-	Yes	Yes	-	Yes
Liu (2020)	No	Yes	Yes	-	Yes	Yes	-	No

Table 8-4 Search methodology of the included studies

Author (Year)	Design of the Included Studies	Level of Evidence	Software	GRADE Use	Sensitivity Analysis
Papakostidis (2008)	RCT and Cohort Study	Level 2	Review Manager	No	Yes
Chen (2012)	RCT	Level 1	Review Manager, STATA	No	Yes
Simmonds (2013)	RCT of IPD, Cohorts, Observational	Level 2	SAS, R	No	Yes
Fu (2013)	RCT of IPD, Cohorts, Observational	Level 2	SAS, version 9.2	No	Yes
Noshchenko (2014)	RCT	Level 1	Not stated	Yes	Yes
Zhang (2014)	RCT	Level 1	Review Manager, R	Yes	Yes
Vavken (2015)	RCT	Level 1	Stata	No	No
Laurie (2016)	RCT IPD	Level 1	SAS	No	Yes
Ye (2017)	RCT	Level 1	Review Manager	No	No
Poorman (2017)	RCT and Cohort Study	Level 2	Review Manager	No	No
Mariscal (2019)	RCT	Level 1	Review Manager	No	No
Han (2019)	RCT and Retrospective	Level 3	Review Manager	Yes	Yes
Liu (2020)	RCT	Level 1	Review Manager	Yes	Yes

Table 8-5 Methodological information of the included studies

Items	Papakostidis (2008)	Chen (2012)	Simmmonds (2013)	Fu (2013)	Noshchenko (2014)	Zhang (2014)	Vavken (2015)	Laurie (2016)	Ye (2017)	Poorman (2017)	Mariscal (2019)	Han (2019)	Liu (2020)
A priori design	0	0	1	1	0	1	1	1	0	1	1	0	0
Duplicate study selection and data extraction	1	1	1	1	1	1	1	0	0	0	0	1	1
Comprehensive literature search	0	0	1	1	1	1	1	0	1	0	1	1	1
Publication status as inclusion criterion	0	0	1	1	0	0	0	1	0	0	0	1	1
List of studies (included and excluded) provided	0	0	1	0	0	0	0	0	1	0	0	0	0
Included study characteristics provided	1	1	1	0	1	1	1	1	1	1	1	1	1
Scientific quality of included studies provided	0	1	1	1	1	1	1	0	1	0	0	1	1
Scientific quality of included studies used appropriately in forming conclusions	1	1	1	1	1	1	1	1	1	0	1	1	1
Appropriate methods to combine findings of studies	1	1	1	1	1	1	1	1	1	1	0	1	1
Likelihood of publication bias assessed	1	1	1	1	1	1	1	0	0	0	0	1	1
Conflict of interest stated	0	1	1	1	1	1	1	1	1	1	1	0	0
Total scores	5	7	11	9	8	9	9	6	7	4	5	8	8

8.5 Discussion

To the best of our knowledge, this is the first systematic review of overlapping meta-analyses examining the use of rhBMP compared to ICBG in spinal fusion surgery. We hope that this study may aide surgeons, and other clinical decision makers, to understand the best available evidence on this topic. In this study, 13 meta-analyses were chosen from a comprehensive literature search. While these studies were published across a relatively similar time period, between 2008 and 2020, they did not contain the same primary trials nor did they draw concordant conclusions concerning the choice of fusion augment in spinal fusion surgery.

One inconsistency noted during the course of this review, was the degree to which rhBMP-2 and rhBMP-7 can be treated as either equivalent or separate medical products throughout the general literature. In this study, 6 meta-analyses dealt with rhBMP-2 only, 2 examined rhBMP-7 only, while 5 examined both rhBMP-2 and 7. While both products share a similar purpose and, at a molecular level, both proteins share extracellular rhBMP Type 1 and Type 2 receptors and intracellular signalling via Smad proteins²⁵², differences remain in their mechanism and formulation. For example, rhBMP-2 plays an important role in endochondral bone formation and knockout models result in a chondrodysplasia phenotype. In contrast, defects of rhBMP-7 result in skeletal patterning deficiencies, predominantly of the ribs, skull and hindlimbs.²⁵³ In addition, both licensed products incorporate different delivery carriers: the InfuseTM / InductOsTM (Medtronic) rhBMP-2 is delivered using an absorbable collagen sponge as a conduit, whereas OP-1 (originally developed by Stryker) uses a collagen putty. These differences limit both the ability to accurately compare the two products and to treat both as a single entity under the umbrella of “BMP”. Of the 5 studies that included both rhBMP-2 and rhBMP-7, 4 performed subgroup analysis to differentiate between the effects of the rhBMP-2 and rhBMP-7 products, however, the study by Noshchenko et al²⁴¹ failed to account for these potential differences.

According to the Jadad decision making algorithm, 2 studies^{223,249} presented the best available evidence on this topic. However, as noted in the results section, the conclusions drawn from these respective studies differs in their recommendation on the use of rhBMP in spine surgery, despite being meta-analyses of the same database of IPD from RCTs. These studies dealt with RCTs of rhBMP-2 versus ICBG only and were drawn from the Yale University Open Data Access (YODA) project. Here, the YODA team invited Medtronic (Minneapolis, Minnesota) to submit data from their primary

RCTs of rhBMP-2 to allow the two study groups to perform independent reanalysis. This data included trial results of the licensed Infuse (dose 1.5g/L) and the unlicensed Amplify (dose 2g/L) products, in addition to the biphasic calcium phosphate (BCP) formulations. Both of these studies drew from the same 12 RCTs for the primary efficacy analysis and included other cohort studies for the safety analysis. Both of these studies found a similar increased rate of fusion of 12% (RR 1.12) at 12 months, with Simmonds²⁴⁹ determining a RR of 1.11 at 24 months compared to the RR of 1.19 determined by Fu²²³. Therefore, according to both of the high quality studies drawn from our review, rhBMP-2 is more efficacious at bone formation than ICBG. For the safety analysis performed by Simmonds et al, overall adverse events were more likely to occur in the rhBMP-2 group. However, these should be interpreted with caution due to the wide confidence intervals secondary to the overall low frequencies of events observed. This is corroborated by the data from the included nonrandomized trials, which determined higher rates of radiculitis, heterotopic bone formation and dysphagia associated with rhBMP-2 use in patients undergoing anterior cervical and discectomy (ACDF) when compared to ICBG. Regarding cancer risk, the overall frequency observed in both groups was low at 2 years, therefore limiting interpretability. However, the cancer risk noted from the RCTs was higher than the ICBG. Fu et al²²³ found no statistically significant differences in adverse event profile between the two groups from the RCT IPD for lumbar fusion procedures. A higher risk profile was noted for BMP in ACDF studies, however a detailed analysis of rhBMP use in the cervical spine is beyond the scope of this review. Again, concerning cancer risk, higher rates were found in the rhBMP-2 group. However, due to low observed numbers, definitive conclusions were difficult to make. Fu et al notes that adverse events are underreported in published RCTs compared to the IPD made available to their team, with publication bias favouring trials in support of rhBMP-2. It is worth noting that neither of these trials performed subgroup analysis between the licensed Infuse graft and the unlicensed Amplify or BCP products. Therefore, from the best available data, it is unclear if rhBMP-2 carries an unfavourable risk profile compared to ICBG, and this should be weighed against the potential morbidity of the bone grafting procedure. As stated above, the morbidity associated with the ICBG harvesting procedure drove the desire to seek biological alternatives, such as the products under investigation in this review. However, current literature is conflicting on the morbidity related to modern iliac crest bone grafting techniques. Banwart et al²¹⁴ stated that severe and major complications can be avoided provided certain procedural refinements, such as limiting subcutaneous

dissection and tension free skin closure, are employed. Moreover, continuous technical refinements have been proposed in modern literature aiming to reduce the harmful sequelae of this procedure²¹⁵⁻²¹⁷. Chronic donor site pain is the most commonly reported adverse event associated with ICBG. However, a recent randomised trial found that patients undergoing ICBG using the lumbar spinal incision approach could not reliably state which iliac crest had been harvested, nor did the donor site have increased pain compared to the contralateral untouched side²¹⁸. These findings have been echoed in other recent studies^{219,220}. Therefore, the overall morbidity of modern ICBG may be reduced compared to the traditional complication rates quoted.

In the reviewed studies which examined rhBMP-7, Ye et al²⁵⁰ determined an inferior fusion rate compared to ICBG for instrumented lumbar fusion. Vavken et al²⁵¹ reported an equivalent fusion rate for rhBMP-7 compared to ICBG. In 3 studies which compared rhBMP-2 and rhBMP-7 as a subgroup analysis, rhBMP-2 achieved higher rates of fusion compared to rhBMP-7, with rhBMP-7 having lower fusion rates compared to ICBG as well. Therefore, current available evidence would suggest that rhBMP-7 in its current form, is not recommend for use in spinal surgery.

The included meta-analyses in this study included 6 publications in spine specific journals (Spine, European Spine Journal, Global Spine Journal, Journal of Spine Disorders and Techniques (now Clinical Spine Surgery)), 4 publications in orthopaedic or trauma journals (Journal of Bone and Mineral Metabolism, Archives of Orthopaedic and Trauma Surgery, Chinese Journal of Traumatology) and 3 publications in general scientific or medical journals (Plos One, Annals of Internal Medicine). The studies identified from the Jadad decision algorithm were published in the Annals of Internal Medicine, a journal specialising in evidenced based medicine, and has the highest impact factor among the included journals. Therefore, high quality orthopaedic spinal evidence can be, and is, published outside of dedicated orthopaedic and subspecialty spinal journals.

While beyond the scope of this study, there are a number of other osteobiologic products brought to market which are worthy of mention in a discussion of bone graft substitutes which surgeons should be aware of. i-Factor Bone Graft, by Cerapaeedics, uses a bovine-derived hydroxyapatite matrix combined with a synthetic 15 amino acid residue (ABM/P-15) which has shown good success in maxillofacial surgery²²⁴. It serves to act as a bioactive attachment factor to replicate the type 1 collagen binding mechanism with osteogenic precursor cells in bone tissue. The product comes terminally sterilised, ready

to use, is stored at room temperature and has a three year shelf life. At 2 year follow-up, in a RCT, ABM/P-15 demonstrated noninferiority to local bone autograft in anterior cervical discectomy and fusion (ACDF)²²⁵. Jacobsen et al²²⁴, in a RCT, demonstrated superior radiographic but equivalent clinical outcomes when comparing ABM/P-15 to a bone allograft in non-instrumented lumbar fusion. In addition, demineralised bone matrix (DBM) products have recently been used in lumbar spinal fusion surgery, namely; Accell Connexus (Citagenix, Laval, Canada), Allomatrix, Grafton DBM, and Osteofil (Medtronic, Minneapolis, Minnesota). These products have shown efficacy as bone graft extenders, when compared to bone graft alone²²².

There are several limitations to this study. Firstly, this study included only meta-analyses published in the English language. In order to succinctly summarise and arrive at the best available evidence, only meta-analyses comprising RCTs were included. However, these RCTs in turn were limited by a two-year follow up and potential industry biases. The included meta-analyses drew from a total of 38 primary studies. These ranged in design from randomised controlled trials to cohort studies. 25 of the included primary trials declared a direct or indirect financial relationship with an industry sponsor for the study. McDonnell et al discusses the potential biases associated with industry sponsored research²⁵⁴. It is well documented that studies which receive financial sponsorship publish more positive or favourable results²⁵⁵. Undoubtedly, industry sponsorship is often required to make these trials possible, however in this review, it is difficult to ascertain the degree of bias that these relationships influence. As discussed above, Fu et al references a potential under-reporting of adverse events when they analysed the IPD for their study.

8.6 Conclusion

This is the first systematic review of overlapping meta-analyses comparing the use of rhBMPs to ICBG in spinal fusion. This systematic review demonstrated inconsistent results among the included meta-analyses. Based on the best available evidence, the use of BMP-2 results in improved fusion rates compared to ICBG. There appears a trend towards a greater adverse risk event profile when using BMP-2. However, it remains difficult to ascertain the significance of this trend. These potential risks should be weighed against the potential morbidity associated with the ICBG procedure.

*Table 8-7 Primary studies included in the meta-analyses. *denotes direct or indirect industry financial support/ conflict of interest declared*

[illegible]

Chapter 9 A Meta-Analysis of the Diagnostic Accuracy of Hounsfield Units on Computed Topography Relative to Dual-Energy X-ray Absorptiometry for the Diagnosis of Osteoporosis in the Spine Surgery Population

9.1 Abstract

9.1.1 Background

The preoperative identification of osteoporosis in the spine surgery population is of crucial importance. Limitations associated with dual-energy x-ray absorptiometry (DXA), such as access and reliability, have prompted the search for alternative methods to diagnose osteoporosis. The Hounsfield Unit (HU), a readily available measure on computed tomography (CT), has garnered considerable attention in recent years as a potential diagnostic tool for reduced bone mineral density (BMD). However, the optimal threshold settings for diagnosing osteoporosis have yet to be determined.

9.1.2 Methods

We selected studies that included comparison of the HU (index test) with DXA evaluation (reference test). Data quality was assessed using the standardised QUADAS-2 criteria. Studies were characterised into 3 categories, based on the threshold of the index test used with the goal of obtaining a high sensitivity, high specificity, or balanced sensitivity-specificity test.

9.1.3 Results

9 studies were eligible for meta-analysis. In the high specificity group, the pooled sensitivity was 0.652 (95% CI 0.526 – 0.760), specificity 0.795 (95% CI 0.711 – 0.859) and diagnostic odds ratio was 6.652 (95% CI 4.367 – 10.133). In the high sensitivity group, the overall pooled sensitivity was 0.912 (95% CI 0.718 – 0.977), specificity was 0.67 (0.57 – 0.75) and diagnostic odds ratio was 19.424 (5.446 – 69.275). In the balanced sensitivity-specificity group, the overall pooled sensitivity was 0.625 (95% CI 0.504 – 0.732), specificity was 0.914 (0.823 – 0.960) and diagnostic odds ratio was 14.880 (7.521 – 29.440). Considerable heterogeneity existed throughout the analysis.

9.1.4 Conclusion

In conclusion, the HU is a clinically useful tool to aide in the diagnosis of osteoporosis. However, the heterogeneity seen in this study warrants caution in the interpretation of results. We have demonstrated the impact of differing HU threshold values on the diagnostic ability of this test. We would propose a threshold of 135 HU to diagnose OP. Future work would investigate the optimal HU cut-off to differentiate normal from low BMD.

9.2 Introduction

Osteoporosis (OP) is a systemic skeletal disease characterised by low bone mass and a progressive microarchitectural deterioration of bone tissue, leading to enhanced bone fragility and a consequent increased risk of fracture²⁹². Osteoporotic fractures result in a considerable socioeconomic and healthcare burden, due to the associated loss of independence and increased mortality rate²⁹³⁻⁶. Furthermore, they are associated with a significant complication profile. Notably, the risk of pseudarthrosis and subsequent mechanical failure has been reported to be as high as 35 % in the setting of osteoporosis, and this is of particular concern in an elderly patient cohort^{61,63,136}. Moreover, the decreased pull-out strength, cut-out and insertional torque, associated with instrumenting osteoporotic patients results in an increased risk of perioperative vertebral fracture and postoperative instrumentation failure¹³⁶. Therefore, the preoperative diagnosis of OP is crucial to ensure timely preoperative optimisation and aide in surgical planning^{126,136}.

The gold standard method to diagnose OP, as defined by the World Health Organisation, is a T-score of - 2.5 (i.e. more than 2.5 standard deviations below the average of a 25 year old adult) obtained by dual-energy X-ray absorption densitometry (DXA)²⁹⁴. However, access to DXA varies considerably internationally¹²¹ and potential sources of error exist through improper patient positioning and scan interpretation²⁹⁵. These limitations have prompted the search for other techniques to diagnose OP. The Hounsfield Unit (HU), described by Schreiber et al²⁹⁶ has recently emerged as a readily available alternative measure of bone mineral density on computed tomography (CT). In recent years, there have been several clinical studies exploring the diagnostic utility of HU. A considerable portion of the spine surgery population undergo CT imaging as routine preoperative planning to accurately assess surgical anatomy for pedicle screw placement, providing an ideal opportunity for OP screening²⁹⁷. Moreover, in the broader

healthcare context, patients commonly undergo CT imaging for a wide spectrum of clinical indications, therefore allowing for opportunistic screening.

As with all diagnostic tests, sensitivity and specificity are inextricably linked and are dependent on the set threshold of the diagnostic test. At present, there is a lack of consensus on the threshold for the HU definition of OP, with varying thresholds used throughout the literature. The purpose of this meta-analysis is to synthesise the literature to-date to ascertain the overall sensitivity, specificity, and diagnostic power of the HU for OP.

9.3 Materials and Methods

9.3.1 Eligibility Criteria

We assessed articles based on the following eligibility criteria. The inclusion criteria included: 1) studies involving human patients/subjects 2) comparison of lumbar spine HU measurement to DXA scores 3) for the diagnosis of osteoporosis or differentiating normal from low BMD. Exclusion criteria were as follows: 1) articles not in the English language 2) studies that employed HU measurements in spinal regions other than the lumbar spine (regarding papers that include sacral levels, only data regarding lumbar levels are included) 3) studies that calculated HU measurements using imaging modalities other than conventional CT (e.g. quantitative (Q)CT) Figure 9-1.

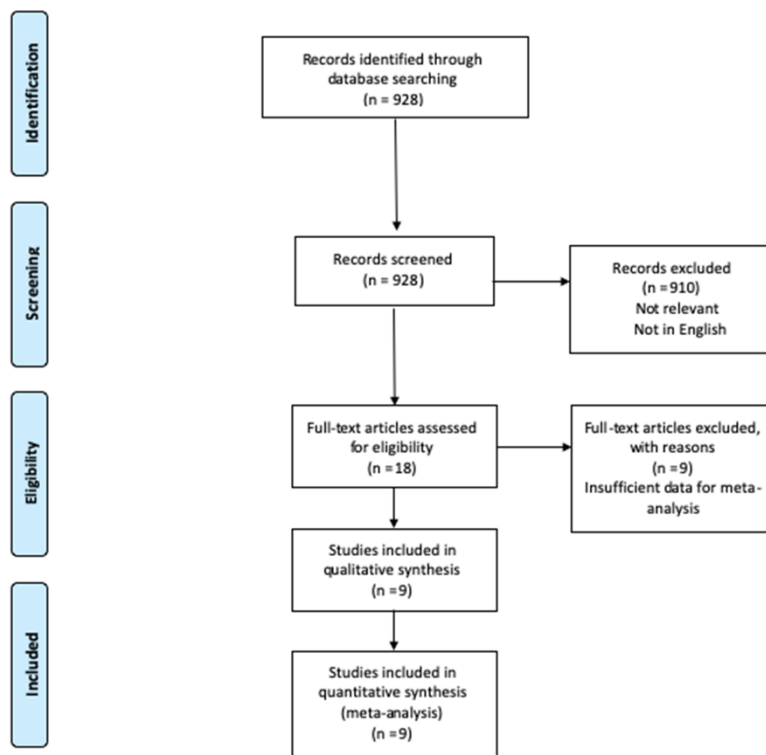


Figure 9-1 PRISMA Flowchart of studies included for meta-analysis

9.3.2 Literature Search

We performed a comprehensive search for eligible articles using the Medline/PubMed database and Cochrane Collaboration to include studies up to and including 25th March 2020. Search terms included “osteoporosis”, “Hounsfield unit”, “spine”, “computed tomography”. Furthermore, the bibliographies of retrieved, full-text articles were screened and the “related articles” feature in PubMed was used to identify further eligible articles. Two authors performed the literature search.

9.3.3 Study Selection

Two reviewers screened titles and abstracts identified in the initial search and performed the full text review of the identified studies.

9.3.4 Methodological Quality Assessment

Data quality was assessed using a standardised procedure as set out in the Quality

Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria²⁹⁸. The QUADAS-2 is a tool for the systematic review of diagnostic accuracy studies and is comprised of four domains: patient selection, index test, reference standard, and flow and timing. In brief, patient selection refers to the methods of patient recruitment, the index and reference test domains relate to how these tests were conducted and interpreted, and finally, flow and timing assesses the interval between reference and index tests.

9.3.5 Statistical Analysis and Data Synthesis

Statistical analysis was performed according to the Cochrane guidelines for diagnostic test accuracy (DTA) reviews²⁹⁹. The purpose of diagnostic investigations is to ascertain whether or not an individual has a particular condition or disease. Statistically, the most common measures used to gauge the accuracy of these tests are sensitivity (the proportion of those with the disease who have an abnormal result) and specificity (the proportion of those without the disease who have a normal result). Diagnostic accuracy studies compare the investigation of interest (index test) to a gold standard (reference) test. Therefore, a DTA review aims to synthesize the results of individual studies comparing the same diagnostic tests, in order to make sense of conflicting results, ultimately to better inform clinical decision making. Here, the index test is the number of Hounsfield units (HU) measured on a region of interest encompassing the lumbar vertebral body on computed tomography, with DXA as the gold standard reference test. However, the HU is a numerical value with varying thresholds used throughout the literature to categorise patients as normal or low BMD / osteoporotic. For accurate comparison between studies, the meta-analysis of DTA requires a common threshold to be used in all index tests. Therefore, we grouped studies in the following categories based off common thresholds used throughout the literature: 1) studies seeking a high specificity (where the HU cut-off

was set at 110 HU) 2) studies seeking a high sensitivity (where the HU cut-off was set at 150 HU) and 3) where studies sought a more balanced sensitivity-specificity approach (where the HU cut-off was set at 135 HU). Where studies used marginally different cut-off values, studies were grouped into the most appropriate category. If studies incorporated more than one cut-off, data was extracted for each appropriate category and examined individually. One study³⁰⁰ separated data by gender, with differing sensitivity and sensitivity values and was therefore, treated as two individual studies for the purposes of analysis.

Statistical analysis was performed using R (version 3.6.3) as described by Shim et al³⁰¹. In brief, the R packages “metaprop” and “metabin” were used for univariate analysis to determine sensitivity, specificity, and diagnostic odds ratio (DOR). A random effects model was chosen for the analysis. In meta-analysis, there are two statistical models to choose from: a fixed effect and a random effects model.³⁰² A fixed effect model is used when it is assumed that there is one true effect size and that all differences in observed effects are due to sampling error. This is appropriate when comparing studies performed on the same population. The random effects model, in contrast, is used when the true effect size may differ between studies. For example, this would be most appropriate when analysing studies involving different populations, with different comorbidities, at different institutions i.e., there is an assumption of inherent heterogeneity. While the difference in effect size may be small, if there is any difference, then a random effects model is most appropriate, and it is this model which is used most commonly in meta-analysis throughout the medical literature. The DOR, a single predictor of overall test performance, can be applied to express the strength of the association between test result and disease and is a common measure used in meta-analysis³⁰³. It is the ratio of the odds of testing positive in those with the disease to the

odds of positivity in those without the disease. The value of the DOR can range from 0 to infinity, with a higher value indicating a better performance. A value of 1 means the test is unable to differentiate between those with and without the disease. For example, if computed tomography (CT) and magnetic resonated imaging (MRI) had DORs of 7 and 12, respectively, when seeking to diagnose a particular condition, the odds of being test positive in those with the disease would be 7 and 12 times higher, respectively, than the odds of being test positive in those without the disease. Therefore, MRI would be the superior investigation of choice. Bivariate analysis was performed using the “reitsma” function of “mada” to generate summary receiving operating characteristic (sROC) curves. Coupled forest plots were used to display the sensitivity and specificity, with their 95% confidence intervals (CI), for all included studies. Forest plots were generated using GraphPad Prism software (version 8.4.1). Visual inspection of individual forest plots was used to identify heterogeneity and bias. The degree of heterogeneity was assessed using Higgins’s I^2 test. The Higgins’s I^2 test quantifies the total variation across studies that is due to heterogeneity rather than chance³⁰⁴. The values of I^2 range between 0% and 100%, with 0% indicating no observed heterogeneity and greater values indicating increasing heterogeneity. While thresholds for the interpretation of the I^2 are debated and should be interpreted in the context of the meta-analysis as a whole, a suggested threshold for their interpretation is 25%, 50% and 75% as low, moderate and high heterogeneity³⁰⁵.

Heterogeneity is typically lower in meta-analyses comparing discrete interventions (e.g. pharmacological intervention versus placebo/ control), whereas heterogeneity is to be expected in a DTA review, for example, Nieves Plana et al³⁰⁶ demonstrated 52% of all DTA Cochrane reviews reported “moderate or extreme” heterogeneity. Summary receiver operating characteristic (SROC) curves were used to display the results of individual studies in a ROC space, each study being plotted as a single sensitivity-false positive rate

point. The SROC curve is the recommended method to represent the performance of a diagnostic test from the data of a meta-analysis.³⁰⁷ The traditional receiver operating characteristic (ROC) curve displays the performance of a diagnostic test by indicating the relationship between true positive rate (TPR) and false positive rate (FPR) of the test across varying thresholds. Varying the threshold will result in changes to the TPR and FPR with the “best cut-off value” as the optimal threshold with the highest TPR and lowest FPR. In the SROC curve of a meta-analysis, each point represents a separate study, and each study contributes an estimate of TPR and FPR. The SROC curve, therefore, intends to represent the relationship between TPR and FPR across the studies being analysed with the “summary estimate” point as the pooled approximation of the overall test performance.³⁰⁷

9.4 Results

9.4.1 Literature Search

The search results are outlined in Figure 9-1. Out of 18 potential articles screened for full-text review, 9 were eligible for meta-analysis^{300,308-315}. Further details of the included studies are outlined in Table 9-1. Study sizes ranged from 50 to 1,867 patients with mean ages ranging from 57.6 to 72.28 years. 4 studies included spinal surgical patient cohorts and the remaining 5 studies included patients undergoing CT abdomen and pelvis for other indications. As indicated in Table 9-1, 4 studies analysed one lumbar level, while 5 studies evaluated multiple lumbar levels. Of those studies that assessed multiple levels^{308-311,313,314}, one study³¹³ reported specific values for the entire lumbar region based on further analysis on the lumbar level HU measurement that shared the highest correlation coefficient with respective DXA values.

Table 9-1 Characteristics of eligible studies

Study	No. of Patients	Patient Population	Mean Age (yr.)	Lumbar Vertebrae Measured	Hounsfield Unit Cut-off
Berger-Groch 2020 ^{308(a)}	50	Spinal surgical patients	72.28 (range 31 – 89)	L4, L5, S1	Normal bone: L4 > 161 HU, L5 > 157 HU, S1 207 HU Osteoporotic bone: L4 < 62 HU, L5 < 58 HU, S1 < 68 HU
Buckens 2015 ^{309 (a, b)}	302	Consecutive patients undergoing CT chest/abdomen with a recent DXA	61 (OP), 57 (Non-OP)	L1	Validation of 160 HU, 110 HU, 80 HU as thresholds
Kim 2016 ^{300 (b, c)}	232	Consecutive patients with CT and recent DXA examination	M = 65.9 +/- 8.5, F = 64.1 +/- 9.9	L1	Male: 136.2 HU = 95% sensitivity, 77.6% specificity and 165.7 HU = 82.5% sensitivity and 90.5% specificity Female: 137.9 HU = 96% sensitivity, 64.4% specificity and 151 HU = 83.9% sensitivity and 86.1% specificity.
Yan-Lin 2018 ^{310 (b, c)}	109	Chinese patients undergoing CT abdomen pelvis for other indications, with recent DEXA	66.8 (range 11 – 91)	L1- L5	diagnosis of OP ≤ 136 HU and exclusion ≥ 175 HU)

Kyung Joon Kim 2019 ^{311 (a)}	331	Spinal surgery patients undergoing QCT, lumbar CT and DEXA	NR	L1-L3	OP $\leq$ 95 $\leq$ normal
Pickhardt 2013 ^{312 (c)}	1867	Patients undergoing abdominal CT and recent DXA	59.2 (+/- 12.5)	L1	135 HU 90% sensitive, 190 HU 90% specific
Cansu 2020 ^{313 (a, b, c)}	111	Consecutive patients with a CT abdomen pelvis and recent DXA	57.6 (16 – 87)	L1-L4	High sensitivity : $\leq$ 170 (sensitivity 88.9%, specificity 25.3%) High specificity: $\leq$ 102 (sensitivity 66.7%, specificity 89.1%) Balanced: $\leq$ 121 (sensitivity 77.8%, specificity 68.9%)
Da Zou 2018 ^{314 (a)}	152	Lumbar spinal surgery patients with recent DXA	58.5 +/- 6.4	L1 – L4	L1: 110 HU, L2 : 100 HU, L3 : 85 HU, L4: 80 HU
Da Zou 2020 ^{315 (a)}	479	Patients over 50 with lumbar degenerative disease	61.8 +/- 6.8	L1	L1 $\leq$ 110 HU

a, b, c denote studies included in a) high specificity (cut-off 110 HU) group b) high sensitivity (cut-off 150 HU) group and c) balanced cut-off (cut-off 136 HU). OP; osteoporosis.

9.4.2 Methodological Assessment

Overall, there was a low risk of bias considered in the methodological quality assessment, as demonstrated in the QUADAS-2 summary (Figure 9-2).

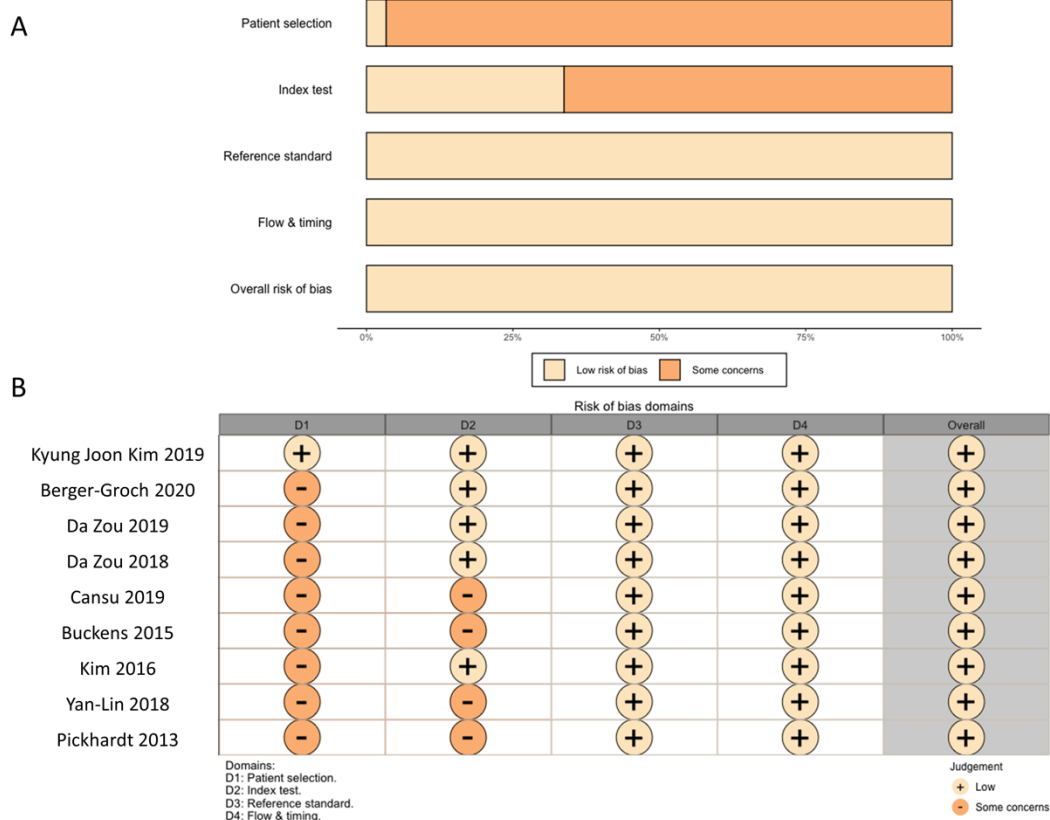


Figure 9-2 A) QUADAS-2 summary figure and B) study specific breakdown of the QUADAS-2 evaluation tool

Studies with a threshold of <110 HU (High Specificity)

6 studies incorporated a threshold designed for high specificity for the differentiation of osteoporosis to non-osteoporosis. The pooled sensitivity was 0.652 (95% CI 0.526 – 0.760), specificity 0.795 (95% CI 0.711 – 0.859) and diagnostic odds ratio was 6.652 (95% CI 4.367 – 10.133). Visual inspection of the forest plots indicates significant study heterogeneity, which is confirmed with an I^2 value of 85%, 81% and 51% for sensitivity, specificity, and diagnostic odds ratio, respectively (Figure 9-3). The summary receiver operating characteristic (SROC) curve, with 95% confidence region is illustrated in (Figure 9-4), demonstrating an area-under-the-curve (AUC) of 0.787.

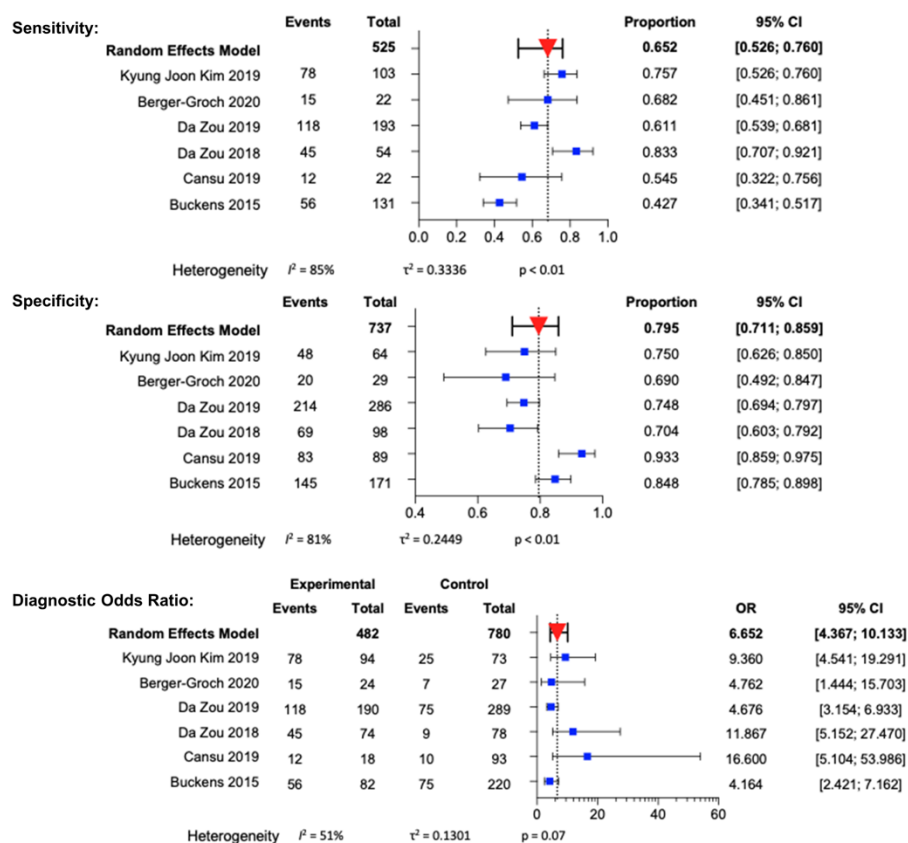


Figure 9-3 Forest plots of sensitivity, specificity and diagnostic odds ratio for studies with a 110 HU cut-off for the diagnosis of osteoporosis.

Summary ROC:

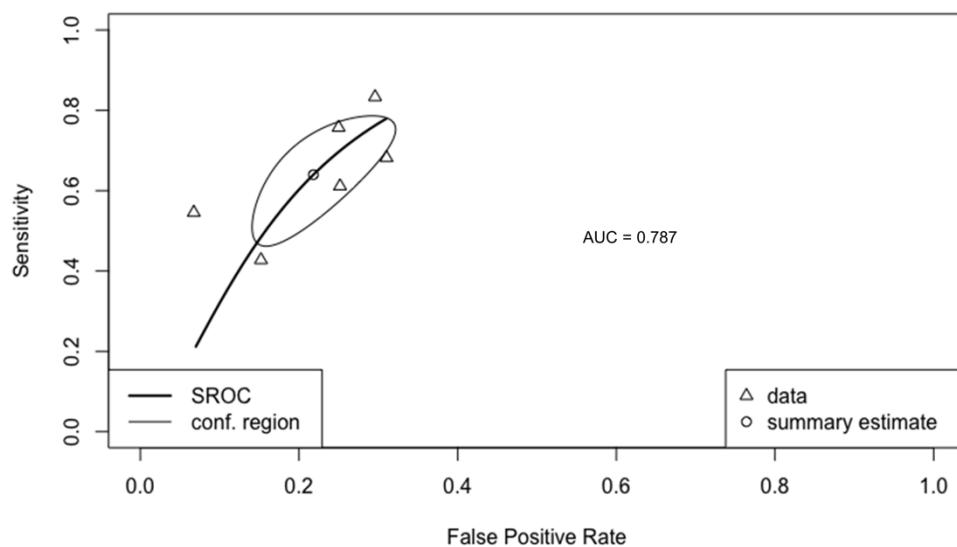


Figure 9-4 Summary receiver operating characteristic (SROC) curve for studies with a 110 HU cut-off for the diagnosis of osteoporosis.

9.4.3 Studies with a threshold >150 HU (High Sensitivity)

5 studies used a threshold of 150 HU to differentiate between normal bone mineral

density (BMD) and low BMD (osteoporotic and osteopenia). The overall pooled

sensitivity was 0.912 (95% CI 0.718 – 0.977), specificity was 0.67 (0.57 – 0.75) and

diagnostic odds ratio was 19.424 (5.446 – 69.275). Heterogeneity is appreciated through

visual inspection of the forest plots, in particular for sensitivity ($I^2 = 96\%$) and diagnostic

odds ratio ($I^2 = 83\%$). The specificity forest plot had less apparent heterogeneity, which

was confirmed with an I^2 value of 26% (Figure 9-3). The SROC curve, illustrated in

Figure 9-4, had an AUC of 0.744.

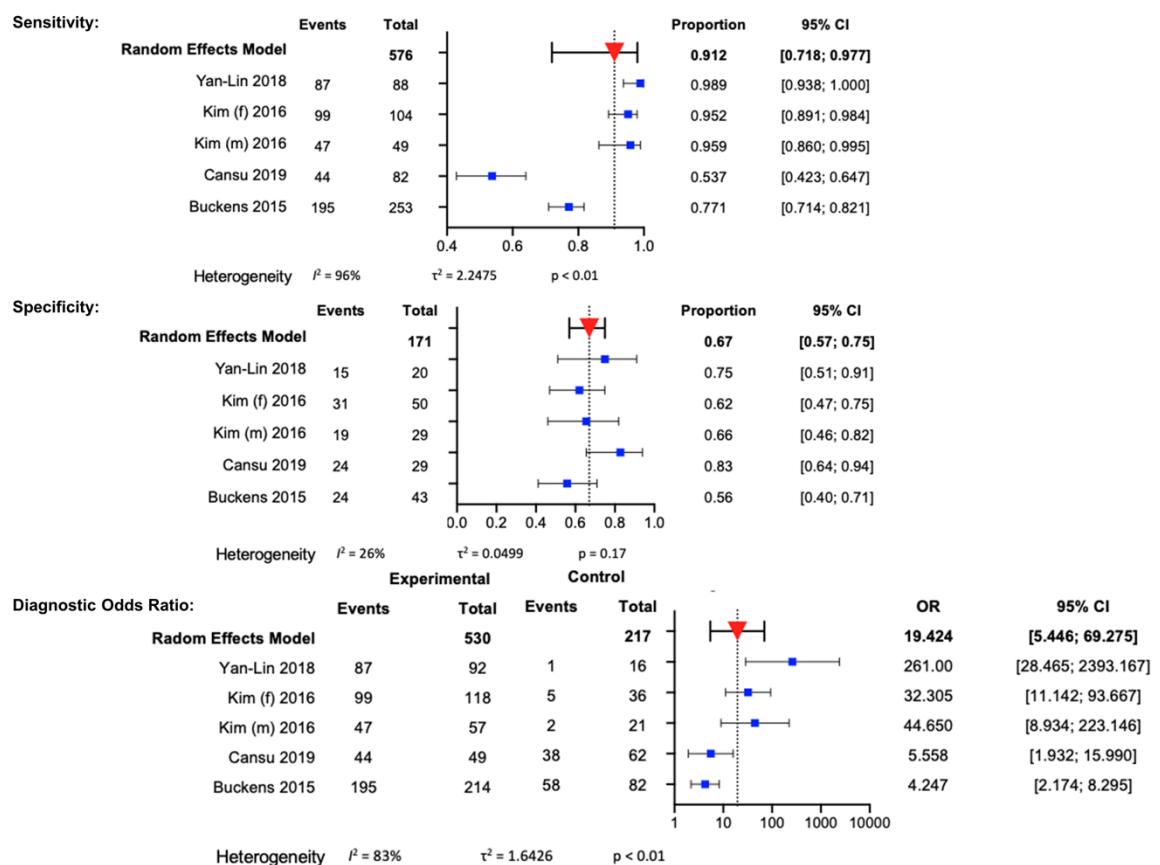


Figure 9-3 Forest plots of sensitivity, specificity and diagnostic odds ratio for studies with a 150 HU cut-off for the diagnosis of normal versus low bone mineral density.

Summary ROC:

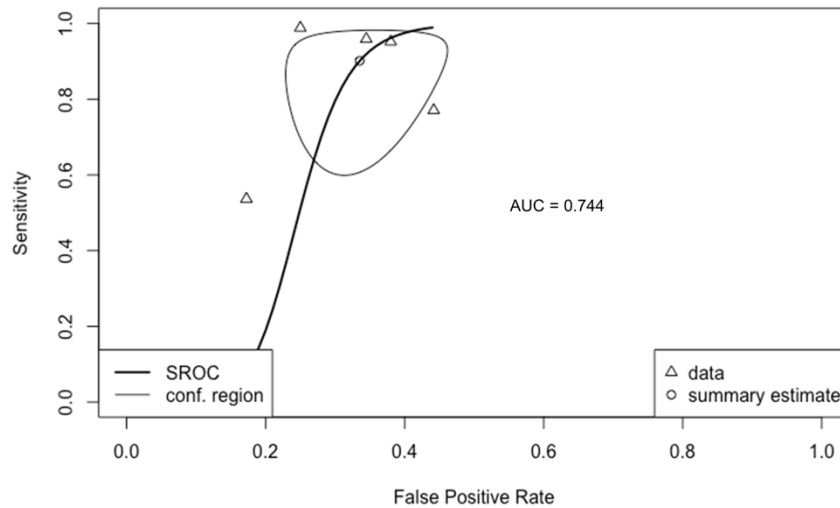


Figure 9-4 Summary receiver operating characteristic (SROC) curve for studies with a 150 HU cut-off for the diagnosis of normal versus low bone mineral density

9.4.4 Studies with a threshold of 135 HU (balanced sensitivity- specificity)

5 studies incorporated a threshold for a more balanced sensitivity-specificity approach for the diagnosis of osteoporosis. The overall pooled sensitivity was 0.625 (95% CI 0.504 – 0.732), specificity was 0.914 (0.823 – 0.960) and diagnostic odds ratio was 14.880 (7.521 – 29.440). Heterogeneity is appreciable on the forest plots and confirmed with I^2 values of 84%, 84% and 63% for sensitivity, specificity and DOR, respectively (Figure 9-5). The SROC curve, with 95% confidence region is illustrated in Figure 9-6, demonstrating an area-under-the-curve (AUC) of 0.831.

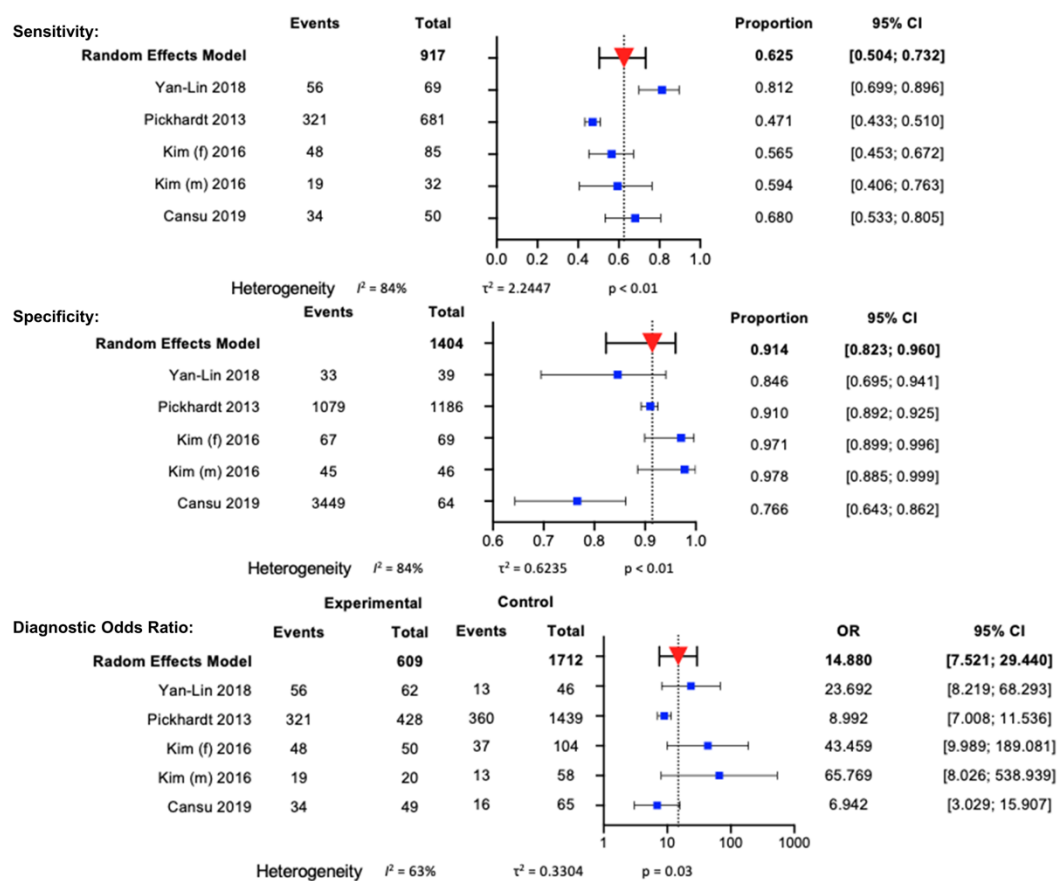


Figure 9-5 Forest plots of sensitivity, specificity and diagnostic odds ratio for studies with a 135 HU cut-off for the diagnosis of osteoporosis

Summary ROC:

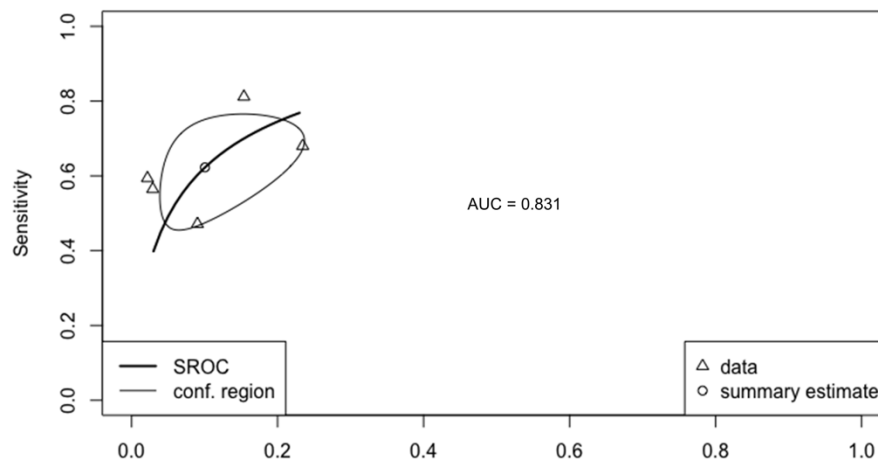


Figure 9-6 Summary receiver operating characteristic (SROC) curve for studies with a 135 HU cut-off for the diagnosis of osteoporosis

9.5 Discussion

In this systematic review, the diagnostic utility of HU for the diagnosis and screening of OP was examined. By employing statistical methods designed specifically for diagnostic meta-analysis, the available evidence surrounding the use of the HU was quantitatively synthesised. Overall, we found that the HU provides clinically useful information regarding BMD determination Table 9-2. For accurate diagnostic test meta-analysis, the threshold between studies must be uniform. Unfortunately, it is not possible to determine pooled thresholds to maximise sensitivity and specificity. Therefore, we divided the evidence into three categories, based on the diagnostic goal of the set threshold: high specificity, high sensitivity, and balanced sensitivity/ specificity. From the methodological assessment, there was, overall, a low risk of bias appreciated by the reviewers. The concerns for potential bias included potential selection bias based on the presence of a recent DXA examination as an inclusion criterion and where the HU

measurement was taken from contrast-enhanced imaging examinations, such as a CT abdomen and pelvis. It is unclear what effect, if any, this bias may have on the results of this study. There were no concerns of bias in regard to the reference DXA test or the flow and timing of the tests.

The group with a threshold cut-off of 110 HU, designed for a higher specificity test, had a pooled sensitivity of 0.652, specificity of 0.795 and DOR of 6.562. Interestingly, the group with a threshold setting of 135 HU had a higher pooled specificity value of 0.914, and DOR of 14.880 with a comparable sensitivity value of 0.625. While significant heterogeneity exists within both study groups, both thresholds provide a clinically useful specificity. A threshold cut-off of 150 HU yielded a pooled sensitivity of 0.912, superior to the thresholds of 110 HU (0.652) and 135HU (0.625). Therefore, one might consider a threshold of 150 HU a clinically effective screening test for osteoporosis.

Considerable work has been published since Schreiber et al²⁹⁶ described the use of the HU as a diagnostic tool for osteoporosis in 2011. However, this is the first meta-analysis on this topic to date. One of the limitations prohibiting the advancement of this technique is the heterogeneity of thresholds used throughout the literature. This heterogeneity is partly due to the differing diagnostic utility of the test (screening and diagnosing). A systematic review by Zaidi et al³¹⁶ in 2018 proposed similar cut-offs to the thresholds used in this analysis: 110 HU for high specificity, 160 HU for high sensitivity and 135 HU for balanced sensitivity-specificity. Wagner et al³¹⁷ utilised this technique to determine that a considerable number of patients undergoing transforaminal lumbar interbody fusion (TLIF) suffered from undiagnosed osteoporosis and low BMD. A cut-off of 150HU was used to determine normal from low BMD, and a cut-off below 112.4 HU

was used to diagnose frank OP versus osteopenia. In this analysis, we determined a cut-off of 150 HU as the most appropriate for a high sensitivity diagnostic approach.

The principle advantage of using the HU for the orthopaedic surgeon is the timely preoperative identification of a patient suffering from low BMD. It is a quick, easy to use and readily available technique with numerous studies reporting excellent inter-observer variability^{296,300,311,318,319}. The preoperative identification of osteoporosis facilitates the medical optimisation of patients perioperatively, such as calcium and vitamin D supplementation and treatment with bisphosphonates or recombinant parathyroid hormone (rPTH)³²⁰. Surgical strategies employed when instrumenting an osteoporotic spine include use of longer constructs and avoiding starting or ending a construct at junctional levels to prevent segmental or junctional failure. One might consider at least 3 fixation points above and below any spinal deformity. Furthermore, when using a long fusion construct, one might consider fixation to the pelvis to maximize construct stability. Anterior column support should be considered to increase load-sharing and minimise the strain on a construct. Pedicle screws can be undertapped to increase insertional torque and pull-out strength. There may also be a role for hydroxyapatite (HA)-coated screws or fenestrated/cement augmented screws to minimise the risk of mechanical failure³²¹.

Another consideration when using this technique relates to the vertebra of interest measured. Mean HU values differ significantly throughout the skeleton, such as the ulna³²², carpal bones³²³, femoral head³¹⁹, femoral neck³¹⁹, talus³¹⁹ and facial bones³²⁴. In the seminal work by Schreiber, mean HU values between the L1-L4 vertebrae were not significantly different²⁹⁶. Pickhardt et al³¹², in the largest series to date on this subject, identified a trend towards differing mean attenuation values between various lumbar vertebrae, with the lowest attenuation at L3. However, this difference between vertebrae was not significant. Berger-Groch et al. demonstrated differing HU values to differentiate

between normal and low BMD in the L4, L5 and S1 vertebrae³⁰⁸. Therefore, we recommend HU measurements to be taken between the L1-4 vertebrae.

Moreover, utilising HU in special populations, such as ankylosing spondylitis (AS), degenerative disease, and scoliosis may provide more accurate measurements of BMD than DXA. AS is a common skeletal inflammatory condition frequently affecting the spine, resulting in both new bone formation and an overall reduction in bone quality³²⁵, which can impair the reliability of DXA³²⁶. Artefactual elevations in calcium content are seen in certain inflammatory conditions of the spine. This does not represent true BMD and can cause falsely elevated readings on DXA which may ultimately go unappreciated by the operator, leading to inaccurate interpretation of certain patients' true BMD³²⁶. Emohare et al³²⁵ demonstrated the utilisation of this technique in a series of 17 patients with AS to good effect. Pappou et al³²⁷ described the positive correlation between falsely elevated BMD scores on lumbar DXA and increasing curve angle in degenerative lumbar scoliosis.

This study has a number of limitations. Firstly, there is a relatively small number of included studies, especially when divided for subgroup analysis, that are predominantly retrospective in nature. Due to this limitation, a sensitivity and heterogeneity analysis was not performed. However, visual examination of the generated forest plots can determine which studies contributed to increased heterogeneity. Secondly, as described above, some studies used marginally different threshold values for each subgroup, we grouped studies into their most appropriate subgroup with the assumption that these differences result in negligible changes in BMD. 5 of the included studies used data generated from CT abdomen and pelvis obtained for other indications, and likely used intravenous contrast. It is not currently known what effect, if any, intravenous contrast has on spinal HU measurement. Thirdly, further analysis regarding variability in

HU measurements due to different tube voltages of specific imaging systems was not possible as it was not consistently listed throughout studies. This is worth noting for further studies as variation in tube voltages has been shown to impact HU measurements³²⁸. There were no funding disclosures or biases which would have influenced the conception, design or interpretation of the study presented herein.

9.6 Conclusion

The HU is a clinically useful tool to aide in the diagnosis of osteoporosis. However, the heterogeneity seen in this study warrants caution in the interpretation of results. We have demonstrated the impact of differing HU threshold values on the diagnostic ability of this test. We would propose a threshold of 135 HU to diagnose OP. Future work would investigate the optimal HU cut-off to differentiate normal from low BMD.

Table 9-2 Summary of Findings Table

General Information		
General Issue	What is the diagnostic performance of computed tomography (CT) derived Hounsfield Unit (HU) in diagnosing osteoporosis (OP)	
Specific Questions	What is the diagnostic performance in distinguishing OP from non-OP?	OP versus osteopenia/normal BMD
	What is the diagnostic performance in distinguishing normal BMD from low BMD?	Normal BMD versus osteopenia/OP
	What is the diagnostic performance when using a balanced threshold to distinguish OP from non-OP?	OP versus osteopenia/normal BMD
Patients	Patients with lumbar spine CT and DXA	
Index tests	CT derived Hounsfield Unit (HU)	
Reference Standard	DXA	
Quality concerns	Overall Judgement	Low risk
	Patient Selection bias	8 studies with some concerns due to retrospective nature of studies
	Index test interpretation bias	4 studies with some concerns due to use of contrast enhanced CT
	Reference test interpretation bias	Low risk

	Flow and timing selection bias	Low risk
Studies which diagnosed osteoporosis at <111 HU		
Studies	6 (1,425 patients)	
Summary results	Sensitivity 0.652 (95% CI: 0.526 – 0.76) Specificity 0.80 (95% CI: 0.71 – 0.86)	
Consequences	In a hypothetical cohort of 1,000 patients (prevalence 40%)	Correctly Classified: 741
		Underdiagnosed: 139
		Over-diagnosed: 120
Studies which diagnosed low BMD at <111 HU		
Studies	4 (754 patients)	
Summary Results	Sensitivity 0.912 (95% CI: 0.718 – 0.977) Specificity 0.67 (95% CI: 0.57 – 0.75)	
Consequences	In a hypothetical cohort of 1,000 patients (prevalence 40%)	Correctly Classified: 767
		Underdiagnosed: 35
		Over-diagnosed: 198
Studies which diagnosed osteoporosis as <135 HU		
Studies	4 (2,319 patients)	
Summary Results	Sensitivity 0.625 (95% CI: 0.504 – 0.732) Specificity 0.914 (95% CI: 0.823 – 0.960)	
Consequences	In a hypothetical cohort of 1,000 patients (prevalence 40%)	Correctly Classified: 798
		Underdiagnosed: 150
		Over-diagnosed: 52

Chapter 10 : The Relationship Between Hounsfield Units, SLIC score, and Failure of One-Stage Anterior Cervical Discectomy and Fusion (ACDF) in Traumatic Sub-axial Cervical Fractures

10.1 Abstract:

10.1.1 Introduction:

Surgical strategies for the management of traumatic cervical fractures include 1-stage anterior (ACDF) or posterior surgery (PCDF), or a circumferential approach. 1-stage surgery is appealing over 2-stage surgery due to reduced intraoperative time and the associated complications. However, certain patients fail a 1-stage anterior approach. The purpose of this study is to identify patient- and injury-specific characteristics associated with failure in 1-stage ACDF to allow for identification of future patients likely to fail a 1-stage ACDF.

10.1.2 Methods:

A retrospective study was performed at our institution from June 22nd, 2016 to March 9th, 2020 to identify patients that suffered traumatic cervical fractures and were managed surgically with a 1-stage ACDF. Data collated included demographic parameters, fracture characteristics, associated injuries, characteristics of fusion surgeries, and appropriate clinical and radiological scores including sub-axial injury classification and severity score (SLIC) and Hounsfield units (HU). Statistical analysis involved t-test of independent means, and z-test of proportions where applicable.

10.1.3 Results:

Twenty-eight patients were identified. Five patients (17.9%) failed the 1-stage ACDF approach and required subsequent posterior instrumentation. Statistically significant parameters that influenced 1-stage ACDF failure were a higher SLIC at presentation (7.00 vs 4.26; $p < 0.01$), a lower HU value on imaging (286.0 vs 412.4; $p = 0.01$), and fractures requiring cervicothoracic junction (CTJ) stabilisation (60.0% vs. 8.7%; $p < 0.01$). When SLIC and HU are assessed together for each patient, 5/5 (100%) patients who failed the 1-stage ACDF approach had a SLIC score of ≥ 5 and a HU score of ≤ 400 , compared to 2/23 (8.7%) of the non-failure cohort ($p < 0.0001$). Similarly, all patients that required posterior instrumentation in the CTJ fixation cohort (3/5; 60.0%) had a SLIC score of ≥ 5 and a HU score of ≤ 400 ($p < 0.01$).

10.1.4 Conclusion:

The definitive management of traumatic cervical fractures remains a surgical challenge. From the results of our study, the authors suggest that surgeons consider patients with $SLIC \geq 5$ and $HU \leq 400$ for primary 2-stage circumferential fixation of traumatic cervical fractures.

10.2 Introduction:

The cervical spine is commonly injured following trauma, particularly motor vehicle accidents and falls from height.^{329,330} Rates of cervical spine injury in trauma vary, but are generally considered to be between 2-4% and increases with age.^{329,331,332} Management of these injuries is complicated and many require procedures to stabilize the spine, but currently, no clear consensus exists on the optimal management of traumatic sub-axial cervical fractures.³³² Conservative management is generally reserved for stable injury patterns and may consist of a rigid cervical orthosis or “Halo Frame” for 6-12 weeks.³³² For unstable injuries, surgical intervention is required to decompress and stabilize the spinal column. Surgical intervention can include 1-stage anterior cervical discectomy and fusion (ACDF), 1-stage posterior cervical discectomy and fusion (PCDF), or a circumferential 2-stage ACDF/PCDF. It has been reported that the 1-stage anterior approaches are associated with fewer infections and increased fusion rates.^{333,334} Nevertheless, both ACDF and PCDF are viable 1-stage procedures for surgical cervical spine stabilization. However, following 1-stage ACDF certain patients require additional posterior instrumentation.

Implant failure is a known complication in many facets of orthopaedic surgery, including total hip arthroplasty, total knee arthroplasty, lumbar spinal fusion, and ACDF.³³⁵⁻³³⁸ Importantly, low bone mineral density has been identified as a risk factor for implant failure and pseudoarthrosis in the same patient populations.³³⁹⁻³⁴¹ Additionally, decreased bone mineral density has been shown to be associated with poor outcomes in lumbar, thoracic, and cervical fusion surgeries.^{339,342-344} Therefore, identifying patient with low bone mineral density is crucial for minimizing post-operative complications. However, many of these studies use dual energy x-ray absorptiometry (DEXA) which is not available for many cervical trauma patients. Additionally, the anatomical differences

between cervical, thoracic, and lumbar vertebrae make extrapolation of lumbar bone mineral density to cervical density extremely difficult. For these reasons, Hounsfield Units (HU) with opportunistic CT imaging has been investigated for correlation to bone mineral density (BMD) as assessed with DEXA. DEXA and HU have been shown to have similar assessments of bone mineral density in the cervical spine at C4, in the lumbar spine, proximal femur, and the glenoid.³⁴⁵⁻³⁴⁹

The Sub-axial Injury Classification and Severity Score (SLIC) is a set of criteria that help the surgeon determine management in sub-axial cervical spine trauma.³⁵⁰ As outlined in Table 1, SLIC can score up to a maximum of 10 points and it is generally considered that injury scores of 1-3pts are managed conservatively, 4 point injuries are managed with a combination of nonsurgical and surgical methods, and injury scores of 5 or greater are managed surgically.³⁵¹ The purpose of this study is to identify if Hounsfield Units (HU) and SLIC scores are related with failure of the 1-stage ACDF approach. Additionally, using the National Spinal Injuries Database we will attempt to identify patient- and injury-specific factors associated with 1-stage ACDF failure.

10.3 Methods

We performed a retrospective analysis between June 22nd, 2016 and March 9th, 2020 at our institution to identify patients that suffered traumatic sub-axial cervical injuries whom were managed surgically with a 1-stage ACDF. Indications such as atraumatic injury mechanism, radiculopathy, degenerative disc disease were excluded from analysis. Twenty-eight patients were identified. Data collected included demographic parameters, fracture characteristics, associated injuries, and fusion surgery characteristics. Neurological injury was assessed pre-operatively with the American Spinal Cord Injury Association Impairment Scale (ASIA). Sub-axial Injury Classification and Severity Scores were captured as outlined per Table 10-1. Hounsfield units were captured from pre-operative CT images as per figure 1. Using the technique described by Schreiber in 2011, C4 was chosen as the level to assess HU for each patient.³⁵² Hounsfield units in the C4 vertebral level has been recently verified as a marker of bone mineral density when compared to DEXA.³⁴⁵ Two authors (H.H. and L.L.) calculated SLIC and HU scores. The mean was taken of the HU values and significant

disagreements were rechecked by the senior author (J.B.). Disagreements in SLIC scoring were rechecked by the senior author. Statistical analysis of the test variables included t-test of independent means and z-tests of proportions where applicable. Failure of the 1-stage ACDF was defined as persistent instability, persistent pain, or unsatisfactory and/or progressive radiological outcomes.

Table 10-1 Sub-axial Injury Classification and Severity Score (SLIC).

Sub-axial Injury Classification and Severity Score	
Parameter	Points
Injury Morphology	
Compression	1
Burst	1
Distraction	3
Translation / Rotation	4
Total	Maximum 4
Integrity of the Disco-Ligamentous Complex	
Intact	0
Intermediate	1
Disrupted	2
Total	Maximum 2
Neurology Status	
Intact	0
Nerve Root Deficit	1
Complete Cord Injury	2
Incomplete Cord Injury	3
Persistent Cord Compression	1
Total	Maximum 4

To evaluate for a potential additive effect of HU and SLIC score we collectively plotted the HU and SLIC score of all individuals. Cut-off scores of SLIC ≥ 5 and HU ≤ 400 were established. SLIC ≥ 5 was chosen due to the previously established suggestion of surgical fixation for these patients.³⁵⁰ The HU cut-off score was established based upon the expert opinion of the senior author. Fisher's exact test was performed to assess the relationship between those with SLIC ≥ 5 and HU ≤ 400 to those without.

10.4 Results

In total, 28 patients were identified that underwent a 1-stage ACDF. Twenty patients were male and eight patients were female. Five patients (17.9%) suffered a 1-stage ACDF failure. Of the five ACDF failures four were male and one female. The mean age of the no-failure cohort was 52.9 (range: 22-85) and was 65.6 (range: 55-79) in the failure cohort ($p=0.08$). Demographic characteristics are summarized in Table 10-2.

Table 10-2 Patient Demographics by Cohort. (M) = Male. (F) = Female

Parameter	No Failure	Failure	P-Value
Cohort (N)	23 (82.1%)	5 (17.9%)	
Mean Age (years)(range)	52.9 (22 – 85)	65.6 (55 – 79)	0.08
Sex	16M / 7F	4M / 1F	0.32

Sub-axial Injury Classification and Severity Score was found to be statistically significant Figure 10-1. Those in the failure cohort had a SLIC score mean of 7.0 versus 4.26 in the no failure cohort ($p < 0.01$). Hounsfield units were also significantly lower in the failure cohort Figure 10-2. Mean Hounsfield Units as assessed with computed tomography through the C4 vertebral body were shown to be significantly lower in the 1-stage ACDF failure cohort than in the non-failure cohort (286.0 vs 412.4; $p = 0.01$). Overall, HU ranged from 180 to 613 and within the failure cohort HU ranged from 228 to 397. Interestingly, there were 14 individuals with $HU > 400$ and 14 with an $HU < 400$. When comparing SLIC scores of individuals above 400 HU (Mean SLIC = 4.93) to individuals below 400 HU (Mean SLIC = 4.57) there is no significant difference ($p = 0.72$). Additionally, comparing HU of individuals with $SLIC \geq 5$ to those with $SLIC < 5$ found no difference between HU in both cohorts. There were 15 patients with a $SLIC \geq 5$ and 13 patients with a $SLIC < 5$. Mean HU in patients with $SLIC \geq 5$ was 393.67 versus 385.40 in patients with $SLIC < 5$ ($p = 0.85$).

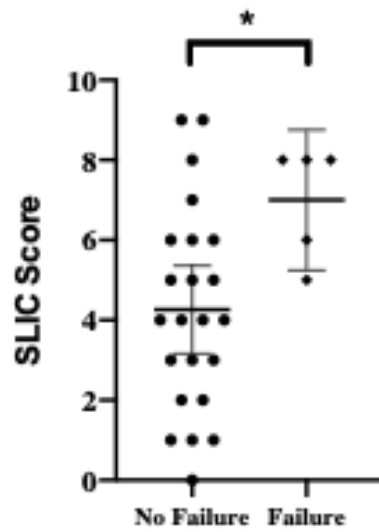


Figure 10-1 Sub-axial Injury Classification and Severity (SLIC) scores for patients of cohorts that were successfully treated with 1-stage ACDF (no failure) and those that required additional posterior stabilization (failure). (*)= $p<0.05$. Note: lines represent mean with 95% confidence intervals (CI) shown

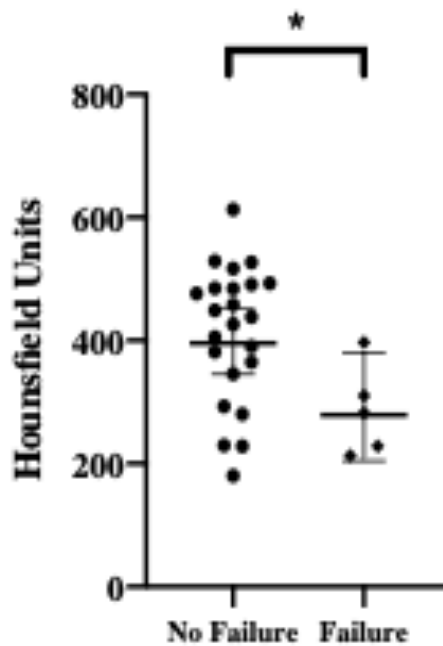


Figure 10-2 Hounsfield Units (HU) for patients of cohorts that were successfully treated with 1-stage ACDF (no failure) and those that required additional posterior stabilization (failure). (*)= $p<0.05$. Note: lines represent mean with 95% confidence intervals (CI) s

Collectively plotting HU and SLIC as seen in Figure 10-3 shows that 7 patients had both a $SLIC \geq 5$ and $HU < 400$, including all 5 patients that failed 1-stage ACDF.

Comparing 1-stage failure rates in patients with SLIC ≥ 5 and HU < 400 (7/28) to the other patients (21/28) revealed a significant relationship ($p < 0.05$).

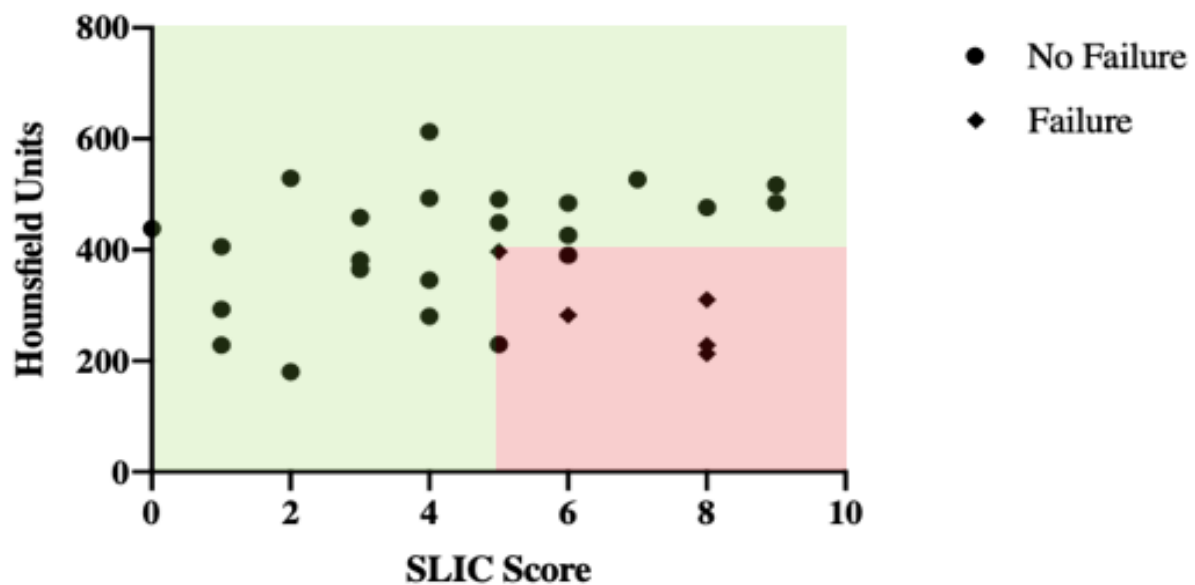


Figure 10-3 Grouped Sub-axial Injury Classification and Severity (SLIC) scores and Hounsfield Unit (HU) scores for each respective patient of cohorts that were successfully treated with 1-stage ACDF (no failure) and those that required posterior stabilisation

There was no statistical relationship between fracture level and failure of 1-stage ACDF. Nor was there any relationship between mechanism of injury and 1-stage ACDF failure. The most common mechanism of injury was a fall from two meters or less (11/28; 39.3%). Neurological deficit was noted in 13/28 patients but was noted statistically associated with failure ($p = 0.251$). ASIA E or no neurological deficit was the most common ASIA grade (15/28; 53.6%). Ligament damage was noted in 20/28 patients with no statistical difference between the failure and no failure cohorts. Interestingly, dislocation was present in 3/5 of the failure cohort and 7/23 no failure patients but this did not reach significance ($p = 0.106$). Clinical injury characteristics are summarized in Table 10-3.

Table 10-3 Clinical Injury characteristics by cohort. (RTA) = road traffic accident; (PLC) = posterior ligamentous complex. (ALL) = anterior longitudinal ligament. (PLL) = posterior longitudinal ligament. (*) = may be multiple ligaments damaged per patient.

Parameter	No Failure	Failure	P-Value
Mean Sub-axial Injury Severity and Classification Score (SLIC)	4.26	7.00	<0.01*
Mean Hounsfield Units (HU)	412.4	286.0	0.01*

Level of Cervical Fracture			
<i>C3 – C5</i>	9 (39.1%)	2 (40.0%)	0.484
<i>C6 – C7</i>	14 (60.9%)	3 (60.0%)	0.484
Mechanism of Injury			
<i>Fall ≤ 2m</i>	8 (34.8%)	3 (60.0%)	0.147
<i>Fall ≥ 2m</i>	3 (13.0%)	1 (20.0%)	0.345
<i>RTA</i>	6 (26.1%)	0 (0%)	0.099
<i>Other</i>	6 (26.1%)	1 (20.0%)	0.389
Fracture Pattern			0.345
<i>Simple</i>	20 (87.0%)	4 (80.0%)	
<i>Complex</i>	3 (13.0%)	1 (20.0%)	
Neurological Deficit	10 (43.5%)	3 (60.0%)	0.251
<i>ASIA A</i>	3	0	
<i>ASIA B</i>	1	2	
<i>ASIA C</i>	1	0	
<i>ASIA D</i>	5	1	
<i>ASIA E</i>	13	2	
Ligament Injury	16 (69.9%)	4 (80.0%)	0.319
<i>PLC</i>	6	1	
<i>ALL</i>	11	3	
<i>PLL</i>	4	2	
Dislocation	7 (30.4%)	3 (60.0%)	0.106

Characteristics related to the surgery such as levels of fusion and those requiring cervicothoracic junction (CTJ) stabilization showed interesting results Table 10-4. There was no statistical relationship between the volume of levels operated on and failure of a 1-stage ACDF. However, there was a statistical relationship between those requiring CTJ stabilization and those not. Of the five failures, 3 (3/5) required CTJ stabilization. And there were only 2 (2/23) patients requiring CTJ stabilization that did not fail the 1-stage approach. SLIC and HU of these patients are available in Table 10-5. Patients requiring CTJ stabilization had no statistical difference in SLIC score (6.33 vs 5.50; $p=0.64$), however, these individuals had significantly lower mean HU (297.33 vs 570.00; $p=0.04$).

Table 10-4 Surgical Characteristics by Cohort. (m) = mean. (*) = $p<0.05$; (CTJ) = cervicothoracic junction.

Parameter	No Failure	Failure	P-Value
Anterior Vertebral Levels Fused	1.2	1.4	0.24
One	19 (82.6%)	3 (60.0%)	0.131
Two	3 (13.0%)	2 (40.0%)	0.076
Three	1 (4.4%)	0 (0%)	0.319
Fusions with CTJ Stabilisation	2 (8.7%)	3 (60.0%)	0.003*
Mean Days to Failure	NA	9.6	
Mean Posterior Vertebral Levels Fused	NA	5.2	

Table 10-5 Hounsfield Units and SLIC Score of patients that required CTJ stabilization.

Patient	SLIC	HU
No Failure		
CTJ Patient 1	4	613
CTJ Patient 2	7	527
Failure		
CTJ Patient 3	5	397
CTJ Patient 4	6	282
CTJ Patient 5	8	213

10.5 Discussion

Following trauma and when required, the cervical spine is often instrumented with a 1-stage anterior cervical discectomy and fusion, a 1-stage posterior cervical discectomy and fusion, or a circumferential 2-stage anterior and posterior approach. Advantages of a 1-stage anterior approach include a reduction in length of surgery, perioperative blood loss, infection risk, and overall length of stay of as a result.³³⁴ Predictably, an increase in aforementioned parameters conveys the disadvantages of a 2-stage approach. Therefore it is crucial that surgeons accurately delineate who requires a 2-stage circumferential operation to stabilize the cervical spine. This presents a controversial debate when cohorts are comprised of elderly patients. We have identified 4 patient and injury related characteristics that can aid the surgeon's decision-making pre-operatively. On our study we demonstrated that patients with increased age, HU <400, SLIC ≥ 5 , and injuries requiring CTJ stabilization are at increased risk of failing 1-stage ACDF.

Identifying patients likely to fail a 1-stage ACDF is crucial as there are significant risks for revision procedures. These patients will be subject to the complications of a revision ACDF, as well as, the complications associated with a circumferential 2-stage procedure increased intra-operative time, blood loss, and surgical cost.³⁵³ A recent trial

showed that patients requiring revision of ACDF for pseudoarthrosis showed that these patients have significantly greater pain-related disability at 1-year follow-up.³⁵⁴ This same study also highlighted significant financial burden related to the revision. Revision following pseudoarthrosis was more than double the cost of the index ACDF (\$18120 vs \$8451; $p<0.01$) and represents a significant and avoidable healthcare expenditure.³⁵⁴

While our study is relatively small, we have identified relationships between 4 important variables that can be assessed pre-operatively to help identify patients requiring 2-stage procedures and preventing failure of 1-stage ACDF. Hounsfield units as assessed through the body of C4 have been shown to be a good proxy of bone mineral density when compared to femoral DEXA scans.³⁴⁵ The authors of this paper suggested two values for assessing BMD. A highly sensitive value of 447 HU to capture 95% of those individuals with low BMD and a highly specific value of 308 HU.³⁴⁵ Our results compliment these findings as all 5 patients that failed 1-stage ACDF were below 447 HU. Additionally, the mean HU of our failure cohort is below the 308 HU threshold (286.00) with 3/5 failures below 308 HU threshold and another patient at 310 HU (Figure 2). In our non-failure cohort, the mean was below 447 HU but well above the 308 HU threshold (412.40). Interestingly, 11/21 non-failure patients were below 447 HU, but only 5/21 were below the 308 HU cut-off.

SLIC scores of 5 or greater suggest that the injury pattern requires surgical intervention.³⁵⁰ However, the clinical applicability of SLIC scoring has been previously questioned.³⁵⁵ We believe we are the first to examine the relationship between SLIC score and 1-stage ACDF failure. This study has highlighted that elevated SLIC scores was significantly associated with failure of 1-stage ACDF. Mean SLIC score (Figure 1) within our failure cohort was 7.0 vs 4.26 in the non-failure cohort ($p<0.01$).

Cervicothoracic junction instability secondary to cervical fractures presents a surgical challenge due to inherent biomechanical properties as the mobile lordotic cervical region transitions towards a more rigid kyphotic thoracic region.³⁵⁶⁻³⁵⁸ Additionally, anatomical differences exist between sub-axial cervical vertebrae. Lateral masses decrease in size from C5-C7, while pedicle masses increase in size, with C7 considered anatomically unique compared to the above sub-axial cervical spine.³⁵⁹ Guidelines regarding the optimal surgical management at the CTJ do not exist, with

advantages and disadvantages to both anterior and posterior 1-stage approaches or a 2-stage anterior-posterior approach. The results of our study highlight the complexities of CTJ stabilization. We identified 3 patients in our failure cohort that required CTJ stabilization and 2 in our non-failure cohort. This relationship was statistically significant ($p=0.003$), however, the sample size is too small to draw any conclusions. Interestingly, HU in the CTJ cohort appears to be important as the 2 non-failures requiring CTJ stabilization had HU of 613 and 527. Whereas the 3 patients requiring CTJ stabilization that failed 1-stage ACDF had HU values of 397, 282, and 213.

While these are interesting results and deserve further investigation, our study is limited due to the small sample size, retrospective nature, and we lack standardization of BMD with a DEXA scan. Additionally, there is heterogeneity with respect to implant constructs and surgeon preference and technique. Also, the results of this study are from a single-centre within Ireland and may not be applicable to the broader global population. As with all retrospective studies our results are subject to confounding and we cannot draw conclusions or extrapolate our results.

The authors encourage larger multicentre prospective studies to evaluate the relationship between SLIC score, Hounsfield Units, cervicothoracic junction stabilization, and failure of 1-stage anterior cervical discectomy and fusion. Based on our results the authors feel that surgeons should consider bone mineral density as assessed with HU, elevated SLIC scores, and the need for CTJ stabilization when determining the optimal surgical plan. Furthermore, biomechanical studies are encouraged to determine the most appropriate number of levels of posterior instrumentation required for CTJ injuries and to evaluate the impact of advancing age on the surgical management of such injuries.

10.6 Conclusion

The definitive management of traumatic cervical fractures remains a surgical challenge with no clear solution. The results of our study suggest that patients with SLIC of ≥ 5 and HU ≤ 400 are at increased risk of failing 1-stage ACDF following traumatic cervical fractures. It is our opinion that surgeons consider these individuals for primary 2-

stage fixation of their injuries. However, further studies are required to thoroughly evaluate the relationship between the identified variables.

Chapter 11 Changing Demographic Trends in Spine Trauma: the Presentation and Outcome of Major Spine Trauma in the Elderly

11.1 Abstract

11.1.1 Introduction:

Major trauma has seen a demographic shift in recent years and it is expected that the elderly population will comprise a greater burden on the major trauma service in the near future. However, whether a similar trend exists in those undergoing operative intervention for spinal trauma remains to be elucidated.

11.1.2 Aims:

To compare the presentation and outcomes of patients ≥ 65 years of age sustaining spine trauma to those < 65 years at a national tertiary referral spine centre.

11.1.2 Methods:

The local Trauma Audit Research Network (TARN) database was analysed to identify spinal patients referred to our institution, a national tertiary referral centre, between 01/2016 and 05/2019. Patients were divided into a young cohort (16 - 64 years old) and an elderly cohort (> 64 years old). No explicit distinction was made between major and minor spine trauma cases. Variables analysed included patient demographics, injury severity, mortality, interventions, mechanism of injury and length of hospital stay.

11.1.3 Results:

A total of 669 patients were admitted of which 480 patients underwent operative intervention for spinal trauma. Within the elderly cohort, this represented 75.3% of cases. Among the younger population, road traffic collisions were the most common mechanism of injury (37.1%), while low falls (< 2 m) (57.4%) were the most common mechanism among the older population. Patients > 64 years old had significantly longer length of stay (21 days [1 - 94] v 14 days [1 - 183]) and suffered higher 30-day mortality rates (4.6% [0 - 12] v 0.97% [0 - 4]).

11.1.4 Conclusion:

Orthopaedic spinal trauma in older people is associated with a significantly higher mortality rate as well as a longer duration of hospitalization. Even though severity of injury is similar for both young and old patients, the mechanism of injury for the older population is of typically much lower energy compared to the high energy trauma affecting younger patients.

11.2 Introduction

The elderly population worldwide has experienced an exponential growth over the past number of decades. In Ireland, 535,393 people, which represents approximately 11.6% of the population, are 65 years old or over, as reported by the Central Statistics Office in 2011³⁶⁰. This figure is due to increase considerably by 2021, with an estimate of 728,606 people. By 2051 this is expected to increase to 1.5 - 1.6 million people^{361,362}. By then, the elderly population will make up around 40% of cases presenting to trauma centres according to the Report of the Trauma Steering Group for Ireland. Data from Major Trauma Audit (MTA) shows a marked increase of the mean age of injured trauma patients from 57 years old in 2014 to 59 years old in 2016³⁶³. A demographic shift in major trauma can be observed today, with the older population living longer and remaining more active and independent^{362,364}. This is accompanied by an age-related decline in motor and cognitive function, which increases this population's susceptibility to trauma³⁶⁴. Due to these developments, trauma centres will face a greater challenge in the prevention and management of morbidity and mortality among geriatric trauma patients in the near future³⁶⁵.

Traumatic injuries are among the leading cause of death and disability worldwide. Major trauma has seen a demographic shift in recent years from the young to the elderly. Current studies demonstrate strong evidence on the increased risk of morbidity and mortality in which elderly trauma patients are currently compared to younger patients³⁶⁶. Mortality rates for this group is higher than in any other age group due to other age-related cofactors³⁶⁷. Among the population-based trauma admissions, almost half result from falls and half of these occur in the elderly^{368,369}. Compared to the total population, the mortality rate for elderly patients who suffer a fall is six times higher³⁷⁰. Moreover, the elderly present with a distinct pattern of injuries, unique anatomical and physiological characteristics as well as age-associated comorbidities³⁷¹.

The recent report from the Major Trauma Audit (2017) has reported 29% of patients admitted for major trauma were reported to have a spinal injury. Spinal surgery represents the second most frequent surgical intervention (10%), followed by surgical intervention for limb injuries³⁶³. Whether a similar trend between age and those undergoing operative intervention for spinal trauma remains to be elucidated. The

purpose of this study was to compare the presentation and outcomes of patients 65 years of age or greater sustaining spine trauma to those under 65 years at a national tertiary referral spine centre. We also wished to investigate whether older patients who suffer spinal injuries have higher mortality rates and length of hospitalization when compared to similar injury severities in younger patients.

11.3 Methods

A retrospective analysis was performed of the Trauma Audit Research Network (TARN) database of patients presenting to the national tertiary referral centre for spinal injuries in the Republic of Ireland, with a catchment area population of approximately 6 million. The TARN database is part of a major collaboration of hospitals from all over England, Ireland, Wales and other parts of Europe, particularly useful in the Major Trauma setting for service evaluation. Submissions to the TARN database are not compulsory. However, it is incentivized and thus have high submission rates especially in Major Trauma Centres in which best practice top-up reimbursements are applicable. An entry is only deemed complete if the required information is provided from presentation/roadside to discharge³⁷². Patients are prospectively enrolled from centres within the United Kingdom and Republic of Ireland. It includes trauma patients who have either a hospital admission of ≥ 72 h due to injury, or a critical care admission, or a transfer to a tertiary/specialist centre or death within 30 days³⁷².

All patients admitted for spinal trauma at our institution between January 2016 and May 2019 were included. Patients who sustained isolated femoral neck or single pubic ramus fractures who are older than 65 years of age were excluded, according to the TARN exclusion criteria. The most widely used definition of major trauma employs the use of the injury severity score (ISS) (3 - 75) which is calculated based on the summation of the squares of the three highest abbreviated injury scale (AIS) scores of injured regions (1- 6) TARN defines major trauma as patients with an ISS score ≥ 15 , however in this study no explicit distinction was made between major and minor spine trauma cases¹⁷(Appendix, Table S1a). Patients who were admitted for a spinal referral and did not undergo a spinal surgical interventions were classified as a conservative intervention. Halo application procedures were classified as a conservative intervention.

Patients were categorized into two subgroups: non-elderly (16 - 64 years old) and elderly patients (≥ 65 years old). The ≥ 65 age range is widely used to represent the elderly group

in the geriatric literature³⁷³⁻³⁷⁶. We examined this cohort of spinal trauma patients, evaluating the effect of age with respect to trauma outcomes by means of injury severity score (ISS), length of stay (LOS) and mortality rates. Demographic, mortality, interventions, mechanism of injury and length of stay in critical care were recorded and analysed via a cross-tabulation system. Surgical interventions were tabulated according to moderate/severe evaluation of trauma (Msev) reported by the TARN database. Prism GraphPad 7.0 software was used to generate the statistical analysis and graphs presented. Cross-tabulation was performed considering the frequency distribution in non-categorical variables such as injury severity and age. Pearson's test was used to evaluate correlations and z-tests were performed to evaluate significant difference between the cohorts.

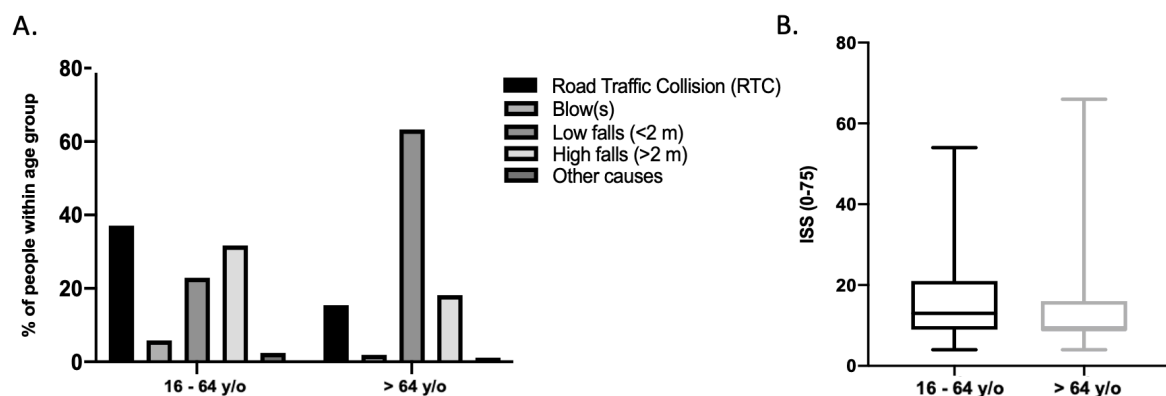


Figure 11-1 (A) Model mechanism of injury in each age group. B) Effect of age in the Injury Severity Score

11.4 Results

Of the 1715 trauma cases admitted, 669 were spinal trauma cases admitted to the national tertiary referral centre for spinal injuries in the Republic of Ireland between November 2015 and May 2019. The data collected from the local TARN database was tabulated for analysis. No patients were excluded based on the exclusion criteria. Variables analysed included demographics, injury severity (ISS), mortality, interventions, mechanism of injury and length of stay (LOS).

Of the 669 patients admitted to the national tertiary referral centre following a spine trauma, the majority were male (64.9%) and the median age of those injured patients was 56.9 years old (15.2 - 99.1) Table 11-1. The median age for the non-elderly group was 40.15 years old (15.2 - 63.9), whereas the median age for the elderly group was 76.3 years old (64.1 - 98.1). The most common mechanism of injury within the non-

elderly group was high-energy accident, such as road traffic collisions (RTC) (37.1%) and high energy falls (>2 m) (31.7%). Among the elderly population, low-energy injuries were the most prevalent mechanism of injury, such as low energy falls (<2 m) (63.3%). The mechanism of injury was significantly different between both age groups for those who suffered road traffic collision (RTC) ($p < 0.00001$), low falls (<2m) ($p = 0.00001$) and high falls (>2m) ($p = 0.001$). While the non-elderly cohort the ISS was 13 [4 - 54], the median ISS for the elderly cohort was 9 [4 - 66]. No significant difference was found in the injury severity score (ISS) between the two age groups ($p = 0.866$) (Figure 11-1, Table 11-1). Furthermore, no significant difference was found in Abbreviated Injury Scale (AIS) between the two age groups ($p = 0.683$). However, the probability of survival was significantly different between the young and elderly patients ($p < 0.001$), with the young population having a mean survival rate of 97.46% and the elderly population having an 89.54% survival rate Table 11-1.

480 patients underwent surgical fixation of their spinal injury, of which 195 (40.6%) patients were in the elderly cohort. In patients ≥ 65 years old, spinal surgical intervention represented 73.7% of admitted cases. While non-elderly patients had a mean length of stay (LOS) of 14 days, the elderly group had a mean LOS of 21 days ($p = 0.0011$). There was no statistical difference between young and elderly cohorts in regards to length of stay in a critical-care unit (33.1% vs 34.7%, $p = 0.3347$). However, patients over 64 years old had a higher 30-day mortality rates (4.6%) when compared to the young cohort (0.97%) ($p = 0.0027$) Table 11-2.

The mechanisms of injury that ultimately required surgical intervention in elderly patients are outlined in Table 11-3. Overall, low falls less than two metres represented the most common mechanism of injury (57.4%) in which surgical intervention was undertaken for patients ≥ 65 years of age. There was a significant difference between age group for those who suffered from low falls (<2 m) ($p < 0.00001$), high fall (>2 m) ($p = 0.003$) and road traffic collisions (RTC) ($p < 0.00001$) Table 11-3. Low falls (<2 m) resulted proportionally higher in the elderly cohort, while high falls (>2 m) and road traffic collisions (RTC) resulting proportionally higher in the non-elderly cohort. Of the 669 patients admitted for a spinal referral, 480 underwent a spinal surgical intervention and 189 were conservatively treated. There was no significant difference found between age groups in relation to surgical intervention ($p = 0.10524$) nor in relation to the conservative interventions ($p = 0.10524$) Table 11-4.

Table 11-1 Demographic and clinical characteristics of the general cohort comparing mechanism of injury, ISS, AIS, and PS.

Demographic and Outcome Variables	General Cohort	Non-Elderly (15 – 64 Years)	Elderly (≥ 65 Years)	P value and effect size for between group comparison
N	669	410 (61.3%)	259 (38.7%)	
Age, median (IQR)	56.9 (15.2 – 98.1)	40.15 (15.2 – 63.9)	76.3 (64.1 – 98.1)	
Sex				
Male	434 (64.9%)	293 (43.8%)	141 (21.1%)	
Female	235 (35.1%)	117 (17.5%)	118 (17.6%)	
Ratio	1.8 : 1	2.5 : 1	1.2 : 1	
Mechanism of Injury				
Road Traffic Collision (RTC)	192 (28.7%)	152 (37.1%)	40 (15.4%)	<0.00001
Blow(s)	29 (4.3%)	24 (5.9%)	5 (1.9%)	0.01352
Low falls (<2m)	258 (38.6%)	94 (22.9%)	164 (63.3%)	0.00001
High Falls (>2m)	177 (26.5%)	130 (31.7%)	47 (18.1%)	0.0001
Stabbing	2 (0.3%)	2 (0.5%)	0	0.25428
Other causes	11 (1.6%)	8 (2.0%)	3 (1.2%)	0.09894
ISS				
Median ISS (IQR)	13 (4 – 66)	13 (4 – 54)	9 (4 – 66)	0.866
Std. deviation	9.48	9.88	8.59	
Severe (ISS ≥ 15)	256 (38.6%)	171 (25.6%)	87 (13%)	
AIS				
Spine	445 (66.5%)	279 (68%)	166 (64%)	0.6835
Probability of Survival (PS)				
Mean PS	94.44 %	97.46%	89.54%	<0.0001

Table 11-2 Interventions, hospitalization and mortality.

	General Cohort (n = 669)	Non-Elderly (15 – 64 Years) (n = 410)	Elderly (≥ 65 Years) (n = 259)	P value and effect size for between group comparison
Surgery				
Head	34 (5.1%)	19 (4.6%)	15 (5.8%)	0.4902
Abdominal	5 (0.7%)	4 (1.0%)	1 (0.4%)	0.3843
Chest	54 (8.1%)	34 (8.3%)	20 (7.7%)	0.779
Spinal	480 (71.7%)	280 (69.5%)	195 (75.3%)	0.105
Limbs	16 (2.4%)	15 (3.7%)	1 (0.4%)	0.007
Multiple	87 (13%)	57 (13.9%)	30 (11.6%)	0.389
Others	2 (0.3%)	1 (0.2%)	1 (0.4%)	0.631
Hospitalisation				
Length of Stay (LOS)	17 (1 – 194)	14 (1 – 194)	21 (1 – 182)	0.001
Stay in Critical Care	226 (33.8%)	136 (33.1%)	90 (34.7%)	0.334
Length of Stay Critical Care	8 (1 – 159)	8 (1 – 129)	10 (1 – 159)	0.089
Mortality				
Deceased	16 (2.4%)	4 (0.97%)	12 (4.6%)	0.003
z-test				

Table 11-3 Comparison of Mechanism of Injury Requiring

Mechanism of Injury	15 – 64 year old Number (N)	≥ 65 year old Number (N)	p-value
Low Falls (<2m)	60 (21.1%)	112 (57.4%)	<0.001
High Falls (>2m)	99 (34.7%)	43 (22.1%)	0.003
Road Traffic Collisions	102 (35.8%)	34 (17.5%)	<0.001
Blow(s)	15 (5.3%)	3 (1.5%)	0.032

Other	9 (3.2%)	3 (1.5%)	0.242
Total	285	195	

Table 11-4 Comparison of surgical and conservative interventions between age groups.

	Total	15 – 64 year old	≥ 65 year old	p-value
Surgical Interventions	480	285 (69.5%)	195 (75.3%)	0.105
Conservative Interventions	189	125 (30.5%)	64 (24.7%)	0.105
Total	669	410	259	

11.5 Discussion

The demographics of major trauma has shifted in recent decades. The average age of trauma patients has been progressively increasing. Moreover, the average age within the elderly cohort is also increasing, with a greater proportion of those at the greater extreme of this age group being admitted for the management of major traumatic injuries.

Combined with the increasing frequency of major trauma, this is leading to higher rates of mortality³⁶². The purpose of this study was to compare the presentation and outcomes of patients >64 years of age sustaining spinal trauma to those <65 years at a national tertiary referral centre for spinal injuries.

Older patients are particularly vulnerable after major trauma. Our findings correlate with Con et al.'s study suggesting major geriatric trauma results in higher hospital admission rates and longer lengths of stay^{377,378}. Longer lengths of stay were associated with more complex morbidity and worse outcomes, with prolonged bed rest associated with deleterious effects in multiple organ systems in the elderly³⁷⁹. In contrast, elderly patients who fell from ladders of lower heights (average of 9.4 ft) sustained different and more severe injury patterns compared to the younger group under 65 years old. However, this did not lead to a significant difference in the mortality rates between age groups³⁷⁷. Injuries occurring at home led to significantly longer hospital stays compared to those happening at work, independent of age³⁷⁸. A one-year study investigating the risk factors for falls among elderly persons in the community, found that

32% of subjects interviewed fell at least once and 24% had serious injuries. Among the risk factors found, 44% were due to the presence of environmental hazards and 10% of falls occurred during acute illness³⁸⁰. Typically, there are many factors associated with falls in the elderly and it is typically associated with longer lengths of stay and higher mortality rates.

The mechanism of injury typically plays an important role in predicting injury severity and mortality. Studies have found that elderly patients admitted due to fall-related injuries were at a higher risk of mortality compared to those involved in motor vehicle collisions³⁸¹. In our study, we found that the majority of elderly patients sustained their injuries from either a high energy or a low energy falls (81.4%), indicating that the most common mechanism of injury for trauma in the elderly population is from falls. This could be associated with a higher mortality rate compared to the younger population whose primary mechanisms of injury were road traffic collisions (RTC) and high falls (68.8%). The primary cause for such falls in the elderly are multifactorial, with elderly patients remaining increasingly more independent and active, resulting in home accidents such as those seen with ladder use which was implicated in many of these accidents^{377,378}.

In the context of low energy falls, studies demonstrate that male trauma patients have a greater 28-day mortality when compared to women with a similar injury severity score (ISS)^{382,383}. Our data demonstrate that patients older than 64 years old have a higher mortality rate when compared to younger patients (4.6% vs 0.97%). These findings are similar to Caterino et al.'s findings where the cut-off age for increased mortality in trauma started at 70 - 74 years old when age groups were stratified by ISS³⁶⁶. Furthermore Wong et al. suggested that the impact of age on mortality starts at 70 years old for females and 55 years old for men which can be attributed to the mortality of male low-fallers³⁸³. This provides an insight into the age at which patients fall into the at-risk group and the age at which fall prevention strategies should be implemented.

In order to understand how the incidence of low energy falls in the elderly population can be a predictor of long-term mortality unrelated to their injuries and a surrogate marker of frailty, the mechanisms of low energy falls need to be explored further³⁸³. Ireland, like all other western societies, is becoming an increasingly aged society, as overall mortality rates decrease and the geriatric population proportion increases^{370,384}. Subsequently, there is expected to be an increase in age-associated diseases. The incidence of fall injuries in the elderly population is expected to increase due to an increasing life expectancy in the world population. Commonly, the injuries

suffered by these patients occur at home without the necessary occupational safety resources. Establishing prevention strategies is the most effective means of reducing the incidence of injury in this group³⁷⁷. Furthermore, the expected increase in healthcare resources required to manage trauma in the elderly should be factored into future public health policies. In 2008, the Health Service Executive (HSE) in the Republic of Ireland released a strategy to prevent falls and fractures in Ireland's aging population. It proposed a multi- agency and multidisciplinary response to prevent low energy falls in Ireland by using the lessons learned from policy changes made in road safety to be used as a model for home safety where most falls occur.

This study is focused on highlighting the foreseen increased burden of the elderly spine trauma in our healthcare system. Further detail into the incidence of different spinal procedures are outside of the scope of this study. Even though not all of the selected patients met the TARN definition of major trauma ($ISS \geq 15$), this study supports the current data of the increased incidence of geriatric trauma as well as giving a generalised perspective of the spine trauma incidence in our healthcare system.

Spine trauma in older people is associated with a significantly higher mortality rate as well as longer length of hospitalization. Even though, injury severity in our study was similar for both younger and older patients, the mechanism of injury for the older population was typically much lower energy compared to the high energy trauma affecting younger patients.

The purpose for this study was to compare both age groups and identify possible trends between age, mechanism of injury and injury severity in order to improve preventative care for the frail population in the context of major spinal trauma. Our data should stimulate increased efforts to improve fall prevention programs for the elderly. Further evaluation on postoperative care and management has yet to be elucidated as mortality, disability and mobility impairment measures are unknown beyond discharge, as well as the effectiveness of fall prevention programs currently implemented.

Chapter 12 Navigation-Assisted Vertebral Body Bone Marrow Aspiration: A Novel Surgical Technique

12.1 Abstract

12.1.1 Introduction

The interest in mesenchymal stem/stromal cells (MSCs) and bone marrow aspirates as cellular therapies in spinal fusion has grown considerably in recent years. Previous studies have described methods to obtain vertebral body bone marrow aspirates using fluoroscopic guidance. However, with the advent of surgical navigation and robotics, these fluoroscopic methods are being used with ever decreasing frequency. Here we describe a novel technique, using surgical navigation technology, to reliably and safely obtain vertebral body bone marrow aspirates.

12.1.2 Methods

Patients undergoing posterolateral spinal fusion or fixation were enrolled to receive transpedicular vertebral body bone marrow aspiration. Here, we describe the step-by-step guide to performing this aspiration using navigation assistance on the O-Arm (Medtronic) platform. For the first four donors, no specific aspiration volume limit was set, and the maximum possible aspiration was taken. However, for donors 5 and 6, a cut-off of a 5ml volume aspiration was imposed. Following aspiration, samples were analysed for cell counts and cultured to assess osteogenic differentiation capacity.

12.1.3 Results

6 patients (3 male, 3 female) were included with a median age of 40.5 (range 20 – 67) years. The median mononuclear cell (MNC) count for the cohort was 2.575×10^7 ($\pm 1.726 \times 10^7$). The mean MNC per millilitre of bone marrow for the first four donors was 2.238×10^6 ($\pm 4.37 \times 10^5$) versus the mean MNC for donors 5 and 6 was 3.44×10^7 ($\pm 1.05 \times 10^7$) ($p = 0.021$). Donor to donor variability was present in terms of the osteogenic capacity of isolated MSCs with donors 1, 2 and 4 demonstrating the highest levels of calcium production. With the exception of donor 5, all donors produced a robust calcium forming response to osteogenic differentiation.

12.1.4 Discussion

We have described a novel method of obtaining vertebral body bone marrow aspirates used to create a biobank of bone marrow MSCs, and further demonstrate the ability of these cells to undergo osteogenic differentiation. This technique ensures safe, reliable aspiration needle placement by utilising navigation technology. In addition, we describe our learning curve, whereby failing to limit the total volume of aspirate lead to significant haemodilution without a concomitant increase in the volume of MNC yielded.

12.1.5 Conclusion

This description for transpedicular aspiration of vertebral bodies can be used for both therapeutic and research purposes. We believe this technique can seamlessly be incorporated into the operative workflow with minimal interruption. The advantages of this technique include precise aspiration needle placement and the ability to harvest bone marrow within the operative field.

12.2 Introduction

The interest in mesenchymal stem/stromal cells (MSCs) and bone marrow aspirates (BMA) as cellular therapies in spinal fusion has grown considerably in recent years. MSCs, a component of bone marrow, are cells that have traditionally been defined by their ability to self-renew, display distinct surface marker expression and differentiate towards bone, cartilage, adipose and neuronal phenotypes²⁹. It is this ability to differentiate into various tissues that has resulted in this widespread interest in the field of regenerative medicine. MSCs have been isolated from numerous tissue types including bone marrow, periosteum, fat, liver, blood and umbilical cord. However, MSCs from different sources vary in their propensity to differentiate into other tissue types^{385,386}. Therefore, for both research and clinical applications, it is prudent to consider and use the most appropriate tissue source for the desired goal.

Traditionally the gold standard to augment bone regeneration and healing is autologous bone graft³⁸⁷. This is most commonly obtained from the iliac crest, however, in both the clinical and research space, this poses considerable limitations. Clinically, donor site morbidity (including chronic pain) with a reported rate of 39%, constitutes a considerable postoperative risk²¹⁴. For biomedical research applications, bone marrow aspirates from the iliac crest are frequently purchased from healthy donors, which carries with it procedural risks and potential complications. One avenue to mitigate these risks and reduce the need for these procedures is to obtain bone marrow from operative sites. Spinal instrumentation (e.g. pedicle screw insertion) commonly necessitates accessing the vertebral body, a source of bone marrow that may be utilised for such applications. Furthermore, Brodano et al³⁸⁸ analysed MSCs harvested from a number of donor sites, including the vertebral body and iliac crest, and found MSCs that were harvested from vertebrae capable of being maintained in culture for longer and more efficient at trilineage differentiation.

The growth of spinal surgery has accelerated rapidly in recent years. Between 2004 to 2015, there were approximately 2.8 million thoracolumbar, lumbar and lumbosacral fusion procedures in the United States for degenerative spinal conditions⁵³. Over this time, the volume of lumbar spinal fusion procedures increased by 63% in 2015 compared to 2004 (60.4 per 100,000 in 2004 to 79.8 per 100,000 in 2015). There was a concomitant increase of 138% in the volume of elective lumbar fusions in those over 65. Overall, the mean spinal fusion failure rate is estimated to be 15%³⁸⁹, with this number projected to be significantly higher in patients with decreased, bone forming potential

such as those with osteoporosis³⁹⁰. Therefore, there is a need to offset these failure rates and a number of bone graft substitutes have been developed in recent years. These include bone allograft, various demineralised bone matrices and synthetic bone grafts²²². In addition, these grafts may be substituted with various peptides in order to increase their efficacy²²⁵. Moreover, attention has turned to using cells, such as MSCs or more generally through BMA, to augment the efficacy of these grafts^{205,207}. The method of obtaining bone marrow aspirate in these cases can vary, from the iliac crest to aspiration of vertebral bodies³⁹¹, resulting in significant variability in terms of cell yield and regenerative potency. Therefore, there is a need to develop a reliable, safe, and efficient aspiration technique to augment to spinal fusion procedures.

The growth of the spinal surgical field coincides with an increase in surgical technology. Previous studies have described methods to obtain vertebral body bone marrow aspirates using fluoroscopic guidance³⁹². However, with the advent of surgical navigation and robotics, these fluoroscopic methods are being used with ever decreasing frequency⁴. Here we describe a novel technique, using surgical navigation technology, to reliably and safely obtain vertebral body bone marrow aspirates. Furthermore, we describe how these aspirates can be used for mononuclear cell culture and expansion, demonstrating their potential as a biobank of MSCs, capable of osteogenic differentiation.

12.3 Methods

Here, we describe a novel method of aspirating vertebral body bone marrow using intraoperative navigation assistance (O-Arm, Medtronic) during pedicle screw implantation. For the purposes of this technical review, six patients were included, enrolled as part of a study with ethical approval from the Institutional Review Board (IRB) of the Mater Misericordiae University Hospital (IRB no. 1/378/2038). The rationale for the study and accompanying risks were explained to the patients and a consent form, approved by the IRB, was signed. Patients with a current history of metastatic neoplastic disease, myelogenous disease or infection were excluded. No exclusion regarding race or gender were applied.

12.3.1 Surgical Technique

Following induction of general anaesthesia, patients were positioned prone on a Jackson radiolucent table or four poster frame. A midline incision exposed the thoracolumbar / lumbar levels to be instrumented, following which a navigation tracker was placed on a spinous process following dissection of the overlying musculature. The O-Arm was introduced to the surgical field and a spin performed for CT reconstruction, following which the surgical navigation instruments were calibrated in space against the fixed reference clamp. Minimally invasive dissection was performed to the pedicle screw entry point and the overlying soft tissue was retracted with a sleeve protector. The entry hole and drill tract are established in the usual manner, under navigation assistance. Following drilling, a navigated Pak needle is brought to the most distal aspect of the drill tract, at which point it is gently placed to the desired centre location of the vertebral body (Figure 12-1). Using a luer lock syringe, an aspirate is taken. This is immediately transferred to a container containing the anticoagulant heparin sodium salt solution (from porcine intestinal mucosa, Sigma-Aldrich H3149) at a concentration of 100 units' heparin per ml bone marrow. The sample is then stored at ambient room temperature until sample processing (Table 12-1).

For the first four donors, no specific aspiration volume limit was set, and the maximum possible aspiration was taken. However, for donors 5 and 6, a cut-off of a 5ml volume aspiration was imposed in an effort to maximise MNC cell yield and limit haemodilution. For the first three donors, a single aspiration from one pedicle screw tract was taken per

vertebra, whereas for the last three donors, an aspiration was taken from both pedicle screw tracts for each vertebra sampled (Table 12-2).

12.3.2 Mononuclear cell expansion and culture

Whole bone marrow was divided into 5ml aliquots and washed in 45ml of PBS (Phosphate Buffered Saline) (Sigma) before centrifugation at 900g for 10 minutes. Pellets were then resuspended in PBS and pooled. An aliquot was diluted 1:1 in 4% acetic acid (Sigma) to lyse the red blood cells present and the mononuclear cell number was counted using a haemocytometer. Cells were seeded at 250,000 cells/cm² in alpha MEM (Sigma) supplemented with 10% foetal bovine serum (FBS) (Biosera), 2% penicillin streptomycin (Sigma) and 1% L-glutamine (Sigma). On day 2 of culture, the media was changed to fresh culture flasks and both flasks topped up with fresh media. On day 5, this was repeated for the flasks seeded on day 2. Following this, culture media was changed every 3-4 days and trypsinised once plastic adherent colonies had grown to 70% confluency. Following trypsinization, cells were seeded at 3000 cells/cm² and grown to 70% confluency. For cell freezing, cells were resuspended in FBS with 10% dimethyl sulfoxide (DMSO) (Sigma) and cooled to -80 Celsius, following which they were stored in liquid nitrogen (-160 Celsius).

12.3.3 Osteogenic Differentiation

Cells were seeded in 12 well plates and grown to 100% confluency, at which point the cells underwent osteogenic differentiation by feeding with osteogenic differentiation media (MEM-alpha medium, 10% FBS, 1% Pen-Strep, 100nM dexamethasone, 10mM sodium beta-glycerophosphate, 2.5mM ascorbic acid) every three days for 21 days. Calcium staining was performed using Alizarin Red Stain at 1% for 20 minutes at room temperature and rinsed in distilled water until the background stain was removed. Calcium stain was then extracted via incubation in 10% acetic acid for 30 minutes under gentle agitation followed by heating at 85°C for 10 minutes. Cell debris was collected by centrifugation at 20,000g for 15 minutes and the pH adjusted to pH 4.1 – 4.5 before measuring absorbance at 405nm.

Table 12-1 Step by step guide to intraoperative transpedicular aspiration using the O-Arm Navigation

Step 1	Surgical preparation and position, prone on a Jackson or four poster frame
Step 2	Surgical approach and dissection
Step 3	Navigation set-up and calibration; mount navigation tracker to fixed bony landmark (e.g. spinous process) and perform O-Arm intraoperative CT reconstruction; calibrate all relevant surgical instruments against the fixed reference clamp including a PAK needle
Step 4	Approach pedicle screw entry point; retract soft tissues with sleeve protector; drill tract in usual manner, under image guidance, to desired area for aspiration
Step 5	Bring navigated PAK needle to tract entry point and under navigation, guide needle to area of interest.
Step 6	Perform aspiration by attaching a Luer-lock syringe to the PAK needle. Quickly transfer the aspirate to a sterile container with 100units in 1mL of heparin sodium salt solution. Alternatively, the syringe can be pre-loaded with the anticoagulant prior to aspiration.
Step 7	Store the sample at ambient room temperature until processing or further use.

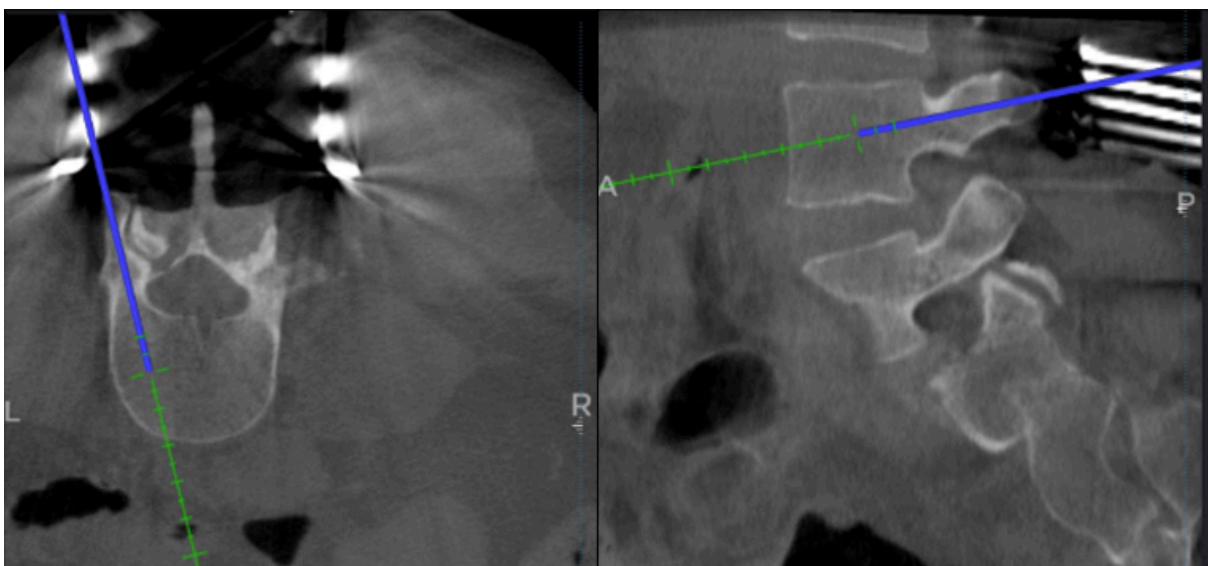


Figure 12-1 Intraoperative navigation image of Donor 1, using O-Arm StealthStation (Medtronic), of navigated Pak needle to desired location within the vertebral body.

12.3.4 Statistical Analysis

Mean values are expressed as mean (+/- standard deviation). For comparison of donor groups an unpaired two-tailed t-test ($\alpha = 0.05$) was performed and in the setting of multiple comparisons a one-way ANOVA was used. Statistical analysis was performed on GraphPad Prism version 9.3.1.

12.4 Results

6 patients (3 male, 3 female) were included in this technical summary. The median age is 40.5(range 20 – 67). 5 patients enrolled underwent posterior spinal fixation for an acute spinal fracture, and 1 patient underwent a lumbar decompression and instrumented posterior spinal fusion for degenerative lumbar canal stenosis. No intraoperative or postoperative complications pertaining to the marrow aspirate or surgical procedure occurred (Table 12-2).

Table 12-2 outlining donor details including past medical history, surgical indication and vertebrae sampled.

Donor	Age	Gender	Past Medical History	Surgical Indication	Vertebrae Sampled
1	34	Male	Nil	Fracture Fixation	L4, L5
2	48	Female	BPAD, Hypothyroid, COPD	Fracture Fixation	T12, L2
3	67	Female	Hypertension, vertigo	Lumbar decompression and PSF	L3, L4, L5
4	20	Male	Nil	Fracture Fixation	L2, L3, L4
5	30	Male	Schizophrenia	Fracture Fixation	L2, L3, L4
6	47	Female	Nil	Fracture Fixation	T12, L1, L2, L3

Aspiration Yields

The median mononuclear cell (MNC) count for the cohort was 2.575×10^7 ($\pm 1.726 \times 10^7$). The mean MNC per millilitre of bone marrow for the first four donors was 2.238×10^6 ($\pm 4.37 \times 10^5$). The mean MNC for donors 5 and 6 was 3.44×10^7 ($\pm 1.05 \times 10^7$) which was a significantly higher yield than donors 1-4 ($p = 0.021$). The mean number of vertebrae sampled was 2.83 (± 0.752) per patient with the mean volume of marrow obtained per vertebrae was 9.588ml. Despite the restriction in volume per aspiration for donors 5 and 6, total MNC yields were higher compared to the previous 4 donors (Figure 12-2).

Osteogenic Differentiation

Donor to donor variability was evident across all donors, with donors 1, 2 and 4 demonstrating the highest levels of calcium production. With the exception of donor 5, all

donors produced a robust calcium forming response to osteogenic differentiation supplements (Figure 12-3).

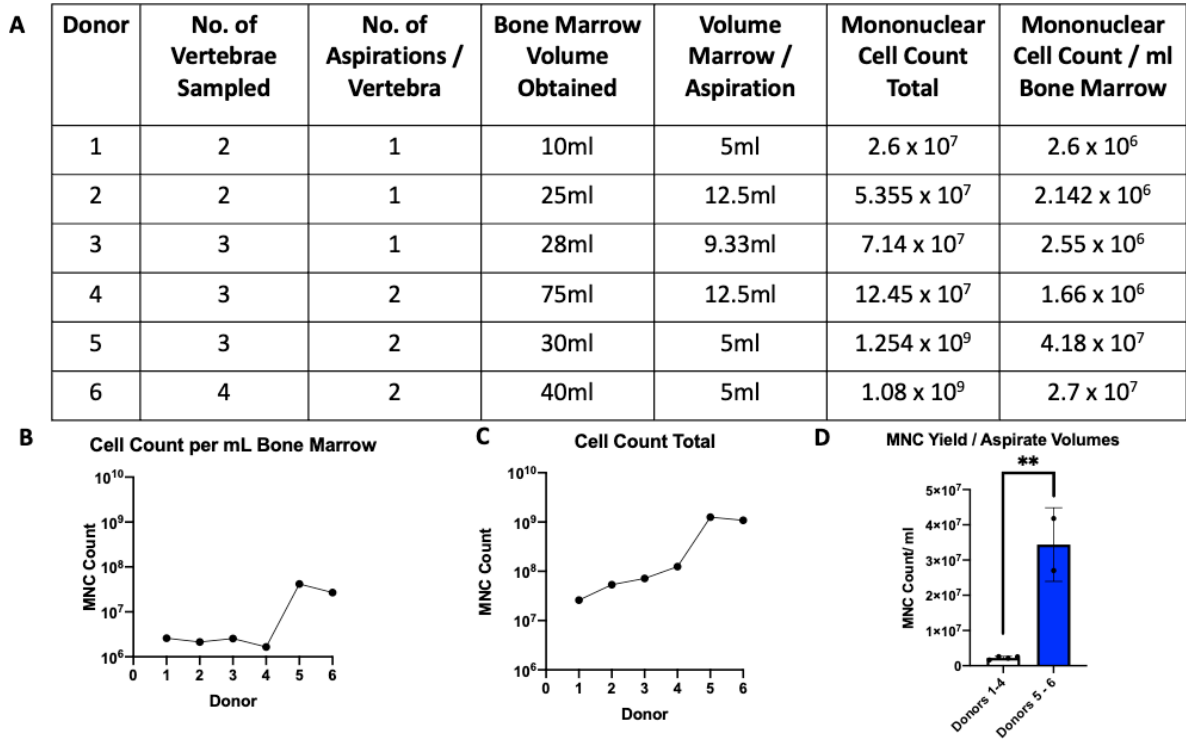


Figure 12-2 Outlining cell count yields and associated aspiration volumes per donor

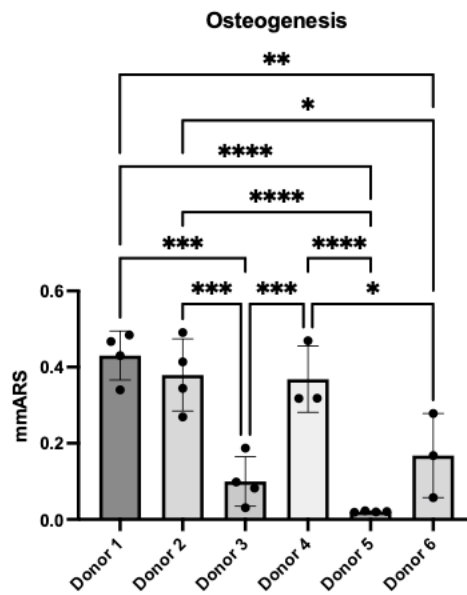


Figure 12-3 Results of osteogenesis assay represented by mm Alizarin Red Stain produced by each donor

12.5 Discussion

Here, we have described a novel method of obtaining vertebral body bone marrow aspirates for use in regenerative medicine or to create a biobank of bone marrow MSCs, and further demonstrate the ability of these cells to undergo osteogenic differentiation. This technique ensures safe, reliable aspiration needle placement by utilising navigation technology. In addition, we describe our learning curve, whereby failing to limit the total volume of aspirate lead to significant haemodilution without a concomitant increase in the volume of MNC yielded.

A number of studies have investigated the utility of the vertebral body (VB) as a source of progenitor cells for research and potential clinical applications. Ahrens *et al.*³⁹³ demonstrated, using human cadaver specimens, that the bone marrow obtained from the VB contained a considerably higher frequency of colony-forming unit-fibroblasts (CFU-Fs) compared to iliac crest bone marrow. Both Risbud *et al.*³⁹⁴ and Brodano *et al.*³⁹⁵ echoed these findings. Risbud *et al.*³⁹⁴ compared bone marrow obtained intraoperatively from lumbar VB and IC in 15 patients. Overall, bone marrow obtained from both locations demonstrated equal bone forming potential with gene expression (Runx-2, collagen type-1, CD44, ALCAM and osteocalcin) and extracellular matrix mineralisation assays. However, the VB marrow demonstrated a higher frequency of CFU-Fs compared to the IC. Moreover, it was noted that the VB CFU-Fs were of smaller size, in addition to

the higher frequency observed. This was hypothesised to be due to an increased differentiation and proliferation rate of the lumbar VB cells, further adding to their clinical potential as an osteogenic tissue. To obtain the VB for the study, Risbud *et al.*³⁹⁴ used a marrow aspiration needle to cannulate the pedicles of the lumbar VB and obtained a 2ml aspirate.

The use of BMA and MSCs as an augment to help promote spinal fusion is increasingly documented in the medical literature. Several studies have used various formulations of BMA or MSCs in combination with a synthetic bone graft substitute to demonstrate comparable fusion rates to ICBG. Kitchel²⁰⁷ in 2006 was probably the first to describe this technique whereby 25 patients underwent a single level posterior fusion with a mineralised collagen synthetic graft in combination with BMA applied unilaterally with ICBG on the contralateral side. Equivalent fusion results were seen with 20/25 (85%) patients achieving fusion on the graft substitute side versus 21/25 (84%) on the ICBG side. In 2014, Hart *et al.*²⁰⁵ compared the addition of BMAC to allograft versus allograft alone in 80 patients undergoing posterolateral fusion. In the experimental arm, patients underwent a BMA from the posterior iliac crests at the start of the operative procedure, which was then added to the allograft. They demonstrated significantly increased fusion rates in the BMAC experimental group. More recently in 2019, Garcia de Frutos²⁰⁶ *et al.* conducted a randomised controlled trial comparing patients undergoing transforaminal lumbar interbody fusion (TLIF) for lumbar degenerative disease to either an allograft with MSCs cultured on same versus ICBG. Here, patients in the experimental group underwent BMA from the posterior iliac crest 21 days preoperatively whereby MSCs were isolated and expanded until 4 hours prior to the procedure, at which point they were then seeded onto the allograft. Overall, patients in the experimental group achieved a higher rate of posterior spinal fusion compared to the ICBG control group. In addition, Neen *et al.*²¹² reported that combining BMA with Healos (DePuy, Synthes), a commercially available type 1 collagen / hydroxyapatite matrix, was easy to use and took less time than obtaining autologous graft. Furthermore, clinical results were not significantly different between the experimental group and the ICBG control and the experimental group was saved from the complications associated from the graft harvest.

This study is limited by design and sample size as a technical description using six patients, without comparative groups. However, we feel it satisfactory to illustrate the technique and our initial learning curve.

12.6 Conclusion

Here, we have described a novel, modern technique for transpedicular aspiration of vertebral bodies which can be used for both therapeutic and research purposes. We believe this technique can seamlessly be incorporated into the operative procedure with minimal interruption to the surgical flow. The advantages of this technique include precise aspiration needle placement and the ability to harvest bone marrow within the operative field. As increasing cell augmented bone grafts come to market, this technique will be instrumental in their real-life application.

Chapter 13 A Comparative Flow Cytometric Analysis of the Human Skeletal Stem Cell and Mesenchymal Stromal Cell in Vertebral Bone Marrow

13.1. Abstract

13.1.1 Introduction

The incidence of spinal fusion surgery is rising dramatically worldwide. In the setting of osteoporosis, spinal fusion surgery is associated with significant technical challenges, fusion failure, morbidity, and healthcare costs. Fusion failure can result due to both mechanical and biological factors. From a biological perspective, mesenchymal stem/stromal cells (MSCs) have long been considered as one of the key cellular precursors in bone formation and have been the subject of a number of pre-clinical and clinical studies exploring their role in spinal fusion. However, the clinical application of MSCs is currently limited by significant variability and rejection issues. This variability could potentially be explained by the heterogeneous nature of MSC samples, with the concept of a MSC undergoing much debate of late. Recently, a purified skeletal stem cell (SSC), with a defined cell lineage, has been identified in adult human long bones. The SSC, and its progeny (Bone-Cartilage-Stromal Progenitor (BCSP), Osteoprogenitor (OP), and Chondroprogenitor (CP)), are defined using distinct cell surface markers, namely podoplanin (PDPN), CD146, CD164, and CD73. Here, we sought to identify the presence of the SSC within human vertebral body (VB) bone marrow and assess the cell surface marker expression of these cells during traditional plastic adherent cell expansion. The purpose of this study is, therefore, to assess the ready applicability of these cells to bone tissue regenerative medicine as putative replacements to the traditionally used MSC.

13.1.2 Methods

Ethical approval was obtained from the Institutional Review Board of the Mater Misericordiae University Hospital, Dublin, Ireland (IRB no. 1/378/2038). Bone marrow aspirates were obtained from the vertebral body bone marrow of patients undergoing posterior spinal fusion. 10mL of each aspirate was committed to initial chemical and mechanical digestion, with subsequent flow cytometric analysis to identify proportions of SSC populations within the marrow isolate. The remaining aspirate from each donor underwent traditional plastic adherent cell culture expansion. Serial flow cytometry was performed at Passages (P) 3 and 7 on the plastic-adherent (PA) mesenchymal stromal cells to identify trends in SSC cell surface marker expression over time. At P3, PA MSCs underwent cell sorting and the SSC, BCSP, OP and CP cell populations were isolated individually from the main MSC fraction. These in turn, were culture expanded to P7 in

tandem with the PA MSC, at which point all populations underwent flow cytometry to assess surface marker expression following expansion.

13.1.3 Results

6 donors were recruited to this study (3 male and 3 female). The hSSC, and related progeny, are present in small quantities in human vertebral bone marrow. The mean percentage populations of the hSSC was 0.054% (+/- 0.099%) within the processed marrow aspirate. The OsteoP (10.870% (+/-6.689%)) population was present in statistically significant greater quantities than the SSC ($p = 0.057$) and BCSP (0.900% (+/-0.434%)) ($p = 0.010$). The proportions of the hSSC populations changes with serial expansion; the hSSC peaked in prevalence at P3 (24.44%) , being present in significantly greater quantities than at P0 (0.054%, $p = 0.03$) and P7 (5.789%, $p = 0.019$). The SSC, and it's progeny, do not maintain their cell surface marker expression during expansion; for the SSC subgroup, at P7 13.04% (+/- 8.478%) of cells were SSC, 5.497% (+/- 4.349%) were BCSP, 11.21% (+/- 8.772%) were OsteoP, 3.941% (+/- 2.947%) were ChondroP and 66.32% (+/- 15.99%) of cells were cell surface marker expression negative.

13.1.4 Discussion

The identification of the hSSC marked a potentially revolutionary discovery in the field of bone regeneration and tissue engineering. In this study, we successfully isolated the hSSC from human vertebral bone marrow from patients undergoing instrumented spinal fusion.

We have explored the potential utility of the hSSC in the spinal surgical setting. We have demonstrated a low observed frequency of hSSCs within a marrow aspirate, therefore necessitating the need for culture expanded cells if cell-based cell therapy is to be considered. Furthermore, we trialled sorting and culture expanding the hSSC from a pool of MSCs and established that the cells lose their surface marker expression over serial expansion.

13.2 Introduction

Spinal fusion surgery has seen marked developments in recent decades, with a concomitant rise in the number of fusion procedures performed annually. In the United States, for example, between 1998 and 2008, the rate of cervical and lumbar fusion rates rose by 90% and 141%, respectively³⁹⁶. With an increase in primary fusion procedures, revision fusion procedures too have risen in incidence³⁹⁶. The rates of fusion failure quoted vary throughout the literature but can be as high as 10% in the cervical spine³⁹⁷, 18% in the thoracic spine³⁹⁸ and 36% in the lumbar spine³⁹⁹. Hospital costs associated with fusion procedures average greater than \$50,000 per admission, with higher costs seen in revision procedures^{53,396}. Increasing rates of fusion and the relatively high rates of revision are intimately related to innovation in the space; improved surgical techniques and technologies have allowed for more procedures to be performed, while the high revision rates have driven the need for further innovation.

Biological augments to spinal fusion are one such area that has seen considerable developments⁴⁰⁰. Bone grafting is an integral component of the fusion procedure, and there have been considerable efforts made to find alternatives to autologous bone graft as the gold standard graft material⁴⁰¹. An ideal bone graft has osteoinductive (provides a biological stimulus that induces cells to enter a pathway of differentiation leading to mature osteoblasts), osteoconductive (promotes bone apposition to its surface functioning in part as a scaffold for bone growth) and osteogenic properties (contains cells capable of differentiating into bone)⁴⁰². A number of potential candidates have been trialled in the clinical arena. Allogenic bone contains osteogenic, osteoinductive and osteoconductive properties but is limited by supply and engraftment issues such as immune rejection⁴⁰³. The recombinant bone morphogenetic proteins (rhBMPs) rose to the fore in the early 2000's and were heralded to be a revolutionary discovery in this space⁴⁰⁴. rhBMPs are bioactive molecules capable of inducing osteogenic differentiation⁴⁰⁵, and combined with a synthetic scaffold, fulfil the osteoinductive and osteoconductive requirements of a bone graft. Indeed, overall the use of rhBMP-2 has led to improved fusion rates in spinal surgery⁴⁰⁶. However, controversies surrounding the original clinical trials supporting their use and potential complication rates have tempered their use in the spinal surgical community. Other proposed alternatives include demineralised bone matrices and synthetic bone grafts, which mainly serve to act as bone graft extenders.

The above synthetic materials lack the cellular or osteogenic component of bone graft. Mesenchymal stem/stromal cells (MSCs) are cells that are present in bone marrow

and are capable of differentiation into bone, cartilage, adipose and a number of other tissue types²⁹. It is, therefore, not surprising, that efforts have been made to harness the potential of these cells in orthopaedic regenerative medicine; as of 2016, there were more than 490 MSC-based clinical trials completed or underway⁴⁰⁷. In spinal surgery, a number of clinical studies have aimed to utilise MSCs in fusion procedures. Notably, a large number of clinical studies use bone marrow aspirate (BMA), harvested intraoperatively, as the source of MSCs²⁰³. Here, the BMA is applied to a scaffold, such as demineralised bone matrix, synthetic bone grafts or allogenic bone etc, with the intent that the principle active ingredient is the MSC. However, MSCs average 1/10,000 cells in an aspirate. Two recent clinical trials have used culture expanded MSCs to overcome this issue of purity with promising results^{206,210}. However, controversy exists within the scientific community over the precise defining criteria of MSCs, and as a result, significant heterogeneity limits the advancement of these cells as potential therapeutic products³⁰.

The description of the human skeletal stem cell (hSSC) in 2018 marks a potential revolutionary discovery⁴⁰⁸, offering the potential to overcome the heterogeneity seen in the MSC population. Here, Chan *et al.*⁴⁰⁸, reported on a multipotent, self-renewing human stem cell, capable of differentiation into bone and cartilage. This cell has distinct cell surface marker expression, namely podoplanin (PDPN)⁺, CD146⁻, CD164⁺ and CD73⁻. Moreover, a lineage tree was described, characterised by varying expression of the aforementioned cell surface markers (Figure X). These cell subsets include a Bone-Cartilaginous-Stromal Progenitor (BCSP / PDPN⁺CD146⁺), a bone / stroma precursor (here, termed “osteoprogenitor” (OsteoP) / PDPN⁻ CD146⁺) and a cartilage precursor (here, termed “chondroprogenitor” (ChondroP) / PDPN⁺ CD146⁻ with CD73⁻ CD164⁻ and CD73⁻ CD164⁺ and CD73⁺ CD164⁻). Since the description of this cell type, a number of further studies have further investigated its properties and potential clinical relevance; Ambrosi *et al.*⁴⁰⁹ isolated SSCs from geriatric fractures in the appendicular skeleton and demonstrated decreased osteochondrogenic capacity compared to SSCs from younger donors; Goodnough *et al.*⁴¹⁰ demonstrated decreased osteogenic capacity in SSCs isolated from a patient who developed a fracture non-union compared to a control who achieved fracture union.

The aim of this study was to 1) isolate the hSSC, and its lineage progeny, from vertebral body bone marrow of patients undergoing spinal fusion or fixation procedures 2) assess the cell surface marker expression of these cells during traditional plastic adherent cell culture. The purpose of this study is, therefore, to assess the ready

applicability of these cells to bone tissue regenerative medicine as putative replacements to the traditionally used MSC.

13.3 Methods

13.3.1 Subjects

Patients admitted for the surgical management of a thoracolumbar spinal pathology (spinal trauma or degenerative spinal conditions) via a spinal fusion or fixation procedure were deemed eligible for enrolment. The rationale for the study and an informed consent form, approved by the Institutional Review Board of the Mater Misericordiae University Hospital, Eccles Street, Dublin 7, Ireland, (IRB reference: 1/378/2038) was signed. Patients with a history of myelogenous disease, an active neoplastic disease or infection are excluded from the study.

13.3.2 Marrow Harvest and Collection

Marrow is aspirated from the thoracolumbar vertebral body (VB) using a marrow aspiration needle (Pak needle), as previously described (see Chapter 12). Under navigated guidance (O-Arm/StealthStation, Medtronic), a marrow aspiration needle cannulates the pedicle of the vertebra and is navigated to the centre of the VB (Figure 13-1). Using a Luer-lock syringe, an aspirate is taken. This is immediately transferred to a container containing the anticoagulant heparin sodium salt solution (from porcine intestinal mucosa, Sigma-Aldrich H3149) at a concentration of 100 units' heparin per ml bone marrow. The sample is then stored at ambient room temperature until sample processing.

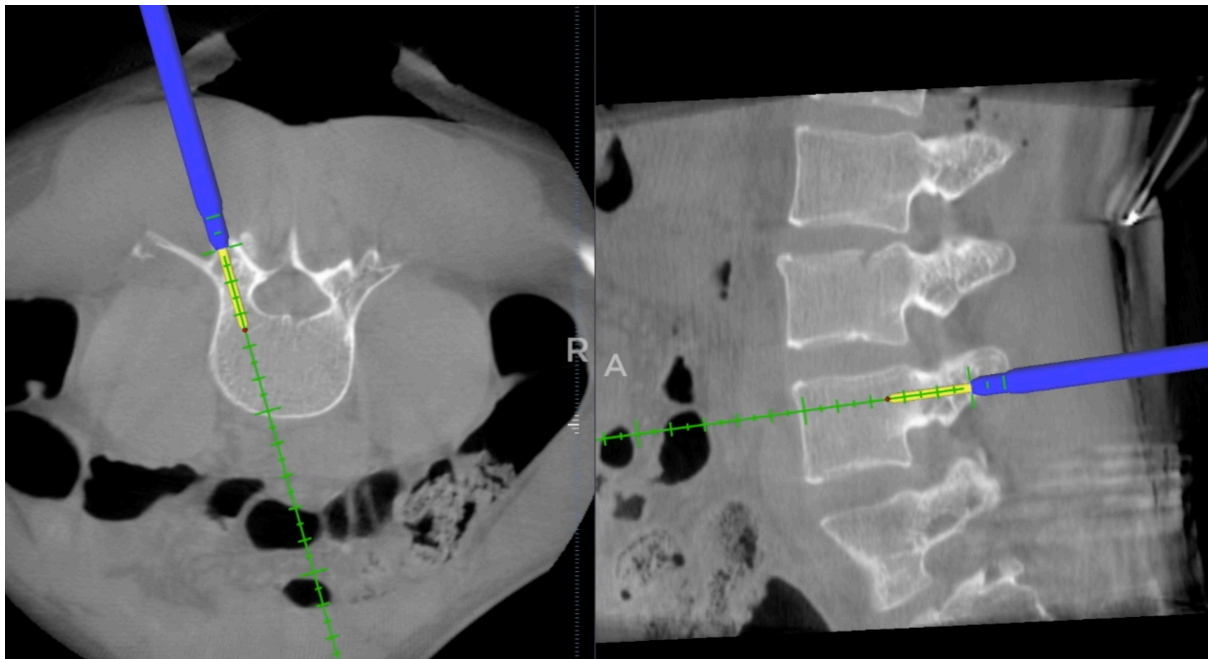


Figure 13-1 Representative image of navigated aspiration needle to the centre of the vertebral body

13.3.3 Experimental Design

The experimental protocol is outlined in (Figure 13-2). Firstly, a portion of the initial marrow aspirate was processed and analysed for the presence of the SSC and related phenotypes using cell surface marker expression (CD146, CD164, CD73 and PDPN). The remaining marrow aspirate was cultured on tissue culture flasks and serially expanded. At Passage 3 (P3), a proportion of the cells were lifted and underwent cell sorting into three populations, namely the SSC, BCSP and Osteoprogenitor, thus leaving 4 experimental groups. These groups underwent serial expansion to Passage 7 (P7), at which point, each group underwent flow cytometric analysis to determine the relative populations within each group following expansion.

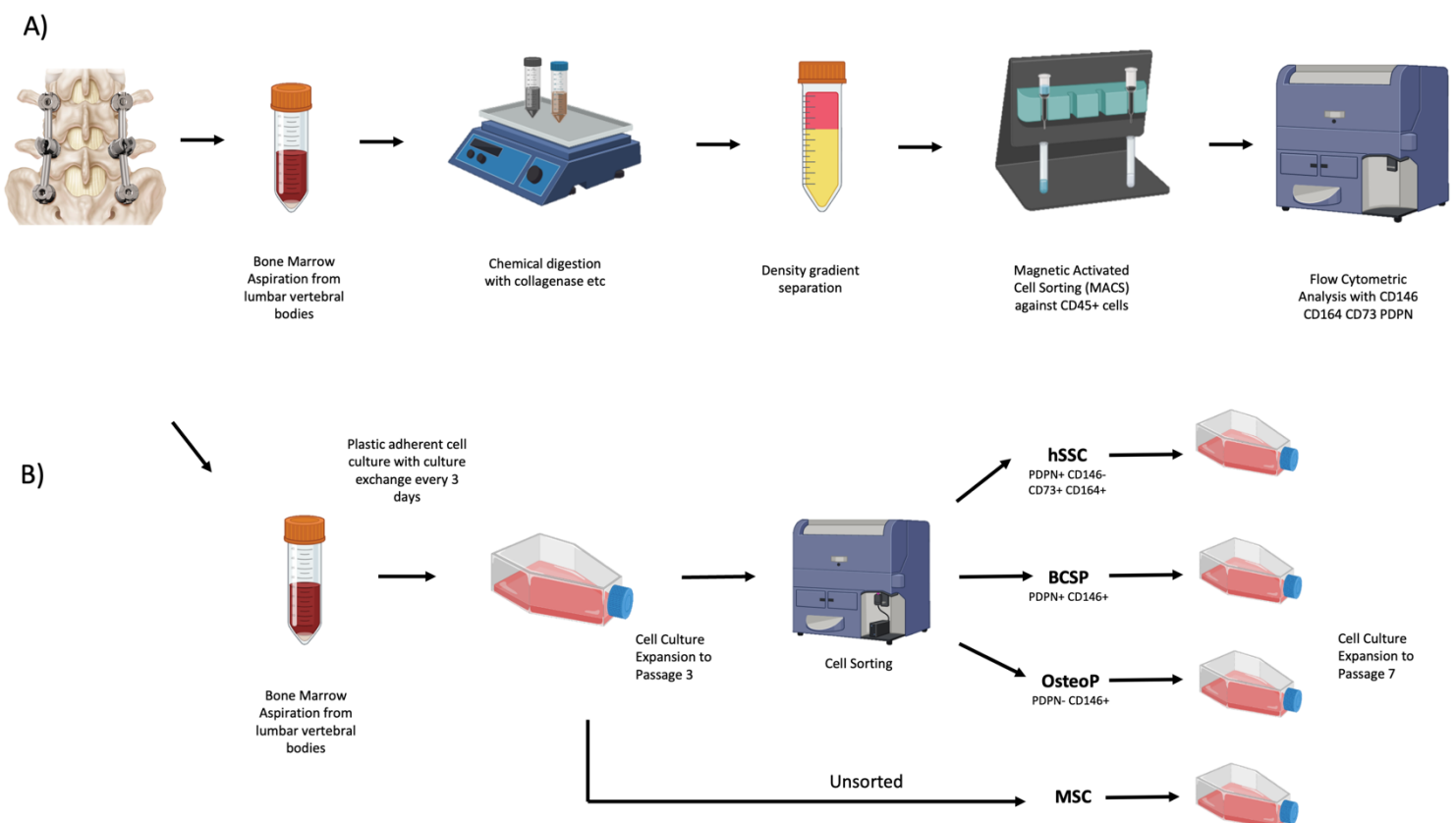


Figure 13-2 outlining experimental design

13.3.4 Bone Marrow Processing for Skeletal Stem Cell Isolation

The protocol for whole bone marrow dissociation is performed as per previously described methods^{408,411}. In brief, the marrow sample was resuspended in 3,000 U/mL type II collagenase (Sigma-Aldrich, Cat#C6885) digestion buffer, which was

supplemented with 100 U/mL DNase I (Worthington, Cat#NC9199796) and incubated at 37°C for 40 minutes under constant agitation. The supernatant was filtered through a 70µm nylon mesh and resuspended with staining media (2% Foetal bovine serum (FBS), in phosphate-buffered saline (PBS)). This digestion was repeated twice more prior to centrifugation at 200g followed by resuspension in staining media.

13.3.5 Magnetic and Fluorescence Activated Cell Sorting (MACS / FACS)

Human skeletal cells were separated from red blood cells (RBCs) and bone dust by density gradient separation using 1:1 Histopaque of density 1.119 g/mL (Sigma-Aldrich, Cat#11191). The buffy coat was collected, washed with staining media, and the resulting cell suspension was depleted of CD45⁺ cells by magnetic-activated cell sorting (MACS) (Miltenyi, Cat#130-045-801). Cells were blocked with 2.5µg of Human BD Fc Block (BD Biosciences, Cat# 564219) for 10 minutes at room temperature. The cells were then stained with fluorochrome-conjugated antibodies against CD73 (Biolegend, Cat#344016), CD164 (Biolegend, Cat# 324808), CD146 (Biolegend, Cat# 342010) and PDPN (ThermoFisher, Cat#17-9381-42). Cell sorting was performed on a BD FACSAria Fusion Cell Sorter (BD Biosciences). Flow cytometry was performed on a BD LSR Fortessa. Gating schemes were established with fluorescence-minus-one (FMO: staining with all fluorophores except one) compensatory (staining of compensation beads (UltraComp eBeads, ThermoFisher, Cat# 01-2222-41) with each fluorophore) controls and negative propidium iodide (PI) staining was used as a measure for cell viability. Based off cell surface marker expression, cells were characterised as either SSC, BCSP, Osteoprogenitor (OsteoP), Chondroprogenitor (ChondroP) or negative for all markers (Figure 13-3).

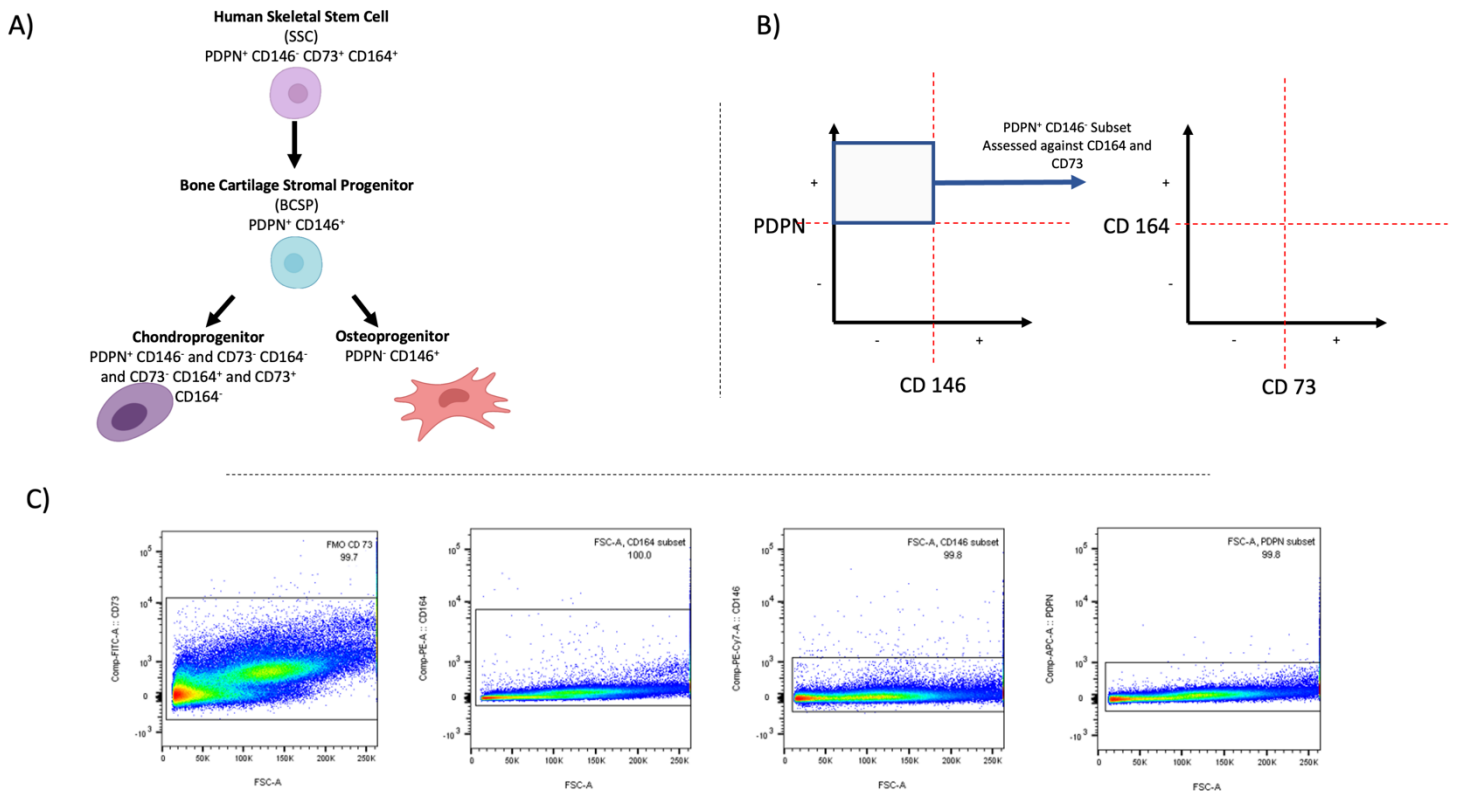


Figure 13-3 A) illustration of human skeletal stem cell lineage tree B) flow cytometry gating strategy c) Fluorescence minus-one (FMO) gating controls.

13.3.6 Cell Culture

Skeletal progenitors isolated from FACS are cultured in MEM-alpha supplemented with 10 % FBS and 1% Pen-Strep in an incubator maintained at 37°C with 5% CO₂.

Mesenchymal stem cells isolated from bone marrow are cultured in medium outlined above (DMEM, 10% FBS, 1% Pen-Strep and bFGF₂). Culture vessels were lifted for analysis or passaging by incubating with trypsin-EDTA solution (Sigma-Aldrich, Cat# T3924).

13.3.7 Statistical Analysis

Statistical analysis was performed using GraphPad (Prism) for MacOS, Version 9.4.1 software. Results are presented as means (+/- standard deviation), unless otherwise stated. Where appropriate, parametric tests including the Student T-test (or Welch t-test where variance is unequal) one way and two way-ANOVA, were performed. Results are considered significant when $p < 0.05$.

13.4 Results

13.4.1 Patient Recruitment

6 patients (3 male, 3 female) were included in this laboratory study. The mean age is 44.3 (range 20 – 67). 5 patients enrolled underwent posterior spinal fixation for an acute spinal fracture, and 1 patient underwent a lumbar decompression and instrumented posterior spinal fusion for degenerative lumbar canal stenosis. No intraoperative or postoperative complications pertaining to the marrow aspirate or surgical procedure occurred Table 13-1.

Table 13-1 Description of demographic and surgical details of included patients

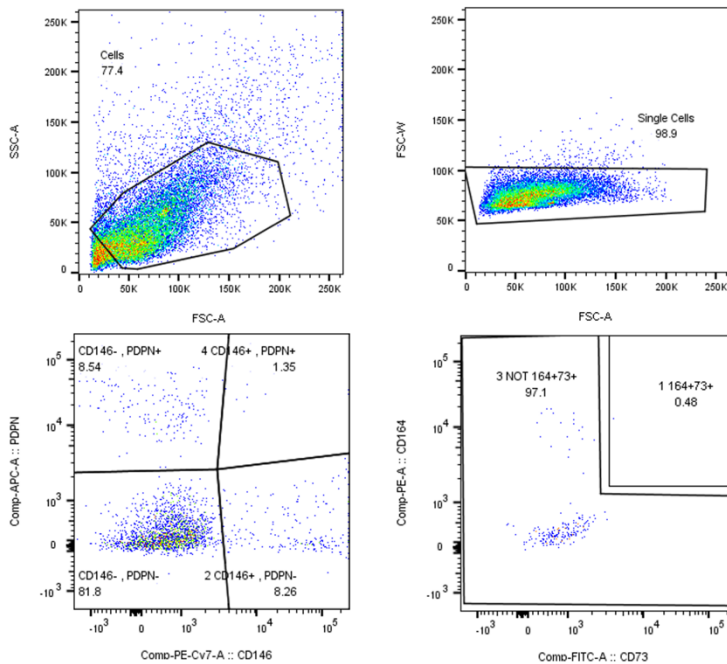
Donor	Age	Gender	Past Medical History	Surgical Indication	Vertebrae Sampled
1	34	Male	Nil	Fracture Fixation	L4, L5
2	48	Female	BPAD, Hypothyroid, COPD	Fracture Fixation	T12, L2
3	67	Female	Hypertension, vertigo	Lumbar decompression and PSF	L3, L4, L5
4	20	Male	Nil	Fracture Fixation	L2, L3, L4
5	30	Male	Schizophrenia	Fracture Fixation	L2, L3, L4
6	47	Female	Nil	Fracture Fixation	T12, L1, L2, L3

13.4.2 The Skeletal Stem Cell, and related progeny, are present in small quantities in human vertebral bone marrow.

The abundance of the hSSC and its progenitor populations was first assessed in fresh marrow aspirates. The mean percentage populations of the various populations within the processed marrow aspirate were as follows Figure 13-4; (SSC 0.054% (+/- 0.099%),

BCSP 0.900% (+/-0.434%), OsteoP 10.870% (+/-6.689%), ChondroP 5.553% (+/-5.208%). The OsteoP population was present in statistically significant greater quantities than the SSC ($p = 0.0057$) and BCSP ($p = 0.010$).

A)



B)

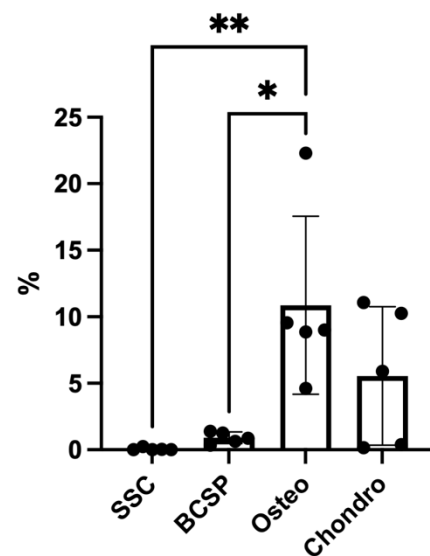


Figure 13-4 Flow cytometric analysis of fresh bone marrow aspirate A) representative flow cytometry gating B) flow cytometry gating strategy c) proportions (%) of individual populations

13.4.3 The proportions of the Human Skeletal Stem Cell populations change with serial expansion

The abundance of the hSSC and progenitor populations was compared at P0 (fresh bone marrow aspirate), P3 and P7 Figure 13-5. At P3, the mean percentage populations of the various populations within the cultured stromal cells were as follows; SSC 24.44% (+/-15.11%), BCSP 8.936% (+/-4.847%), OsteoP 10.41% (+/- 6.793%), and ChondroP 0.131% (+/- 0.147%). In contrast to the fresh marrow aspirate, the proportion of the hSSC was statistically greater than the BCSP ($p = 0.0425$) and ChondroP ($p = 0.016$). At P7, the mean percentage populations of the various populations within the cultured stromal cells

were as follows; SSC 5.789% (+/- 4.433%), BCSP 5.362% (+/- 4.222%), OsteoP 24.49% (+/- 23.56%), and ChondroP 1.122% (+/- 0.679%). At P7, only the OsteoP was present in statistically significant greater quantities than the ChondroP ($p = 0.0392$). No other statistically significant differences were seen.

The proportions of each population fluctuated during serial expansion. The SSC peaked in prevalence at P3 (24.44%), being present in significantly greater quantities than at P0 (0.054%, $p = 0.03$) and P7 (5.789%, $p = 0.019$). The BCSP increased in prevalence at P3 (8.936%) compared to P0 (0.9%, $p = 0.01$). It maintained its expression at P7, with no statistical significance seen between P3 and P7 ($p = 0.308$). In contrast, the ChondroP's expression peaked at P0 (5.553%), before a marked decrease to 0.131% at P3 ($p = 0.023$). The OsteoP maintained a consistent expression across expansion with no statistical differences seen between groups. Taking the SSC Family as a whole, no significant differences were seen in expression across expansion, however a trend could be appreciated towards peaked expression at P3.

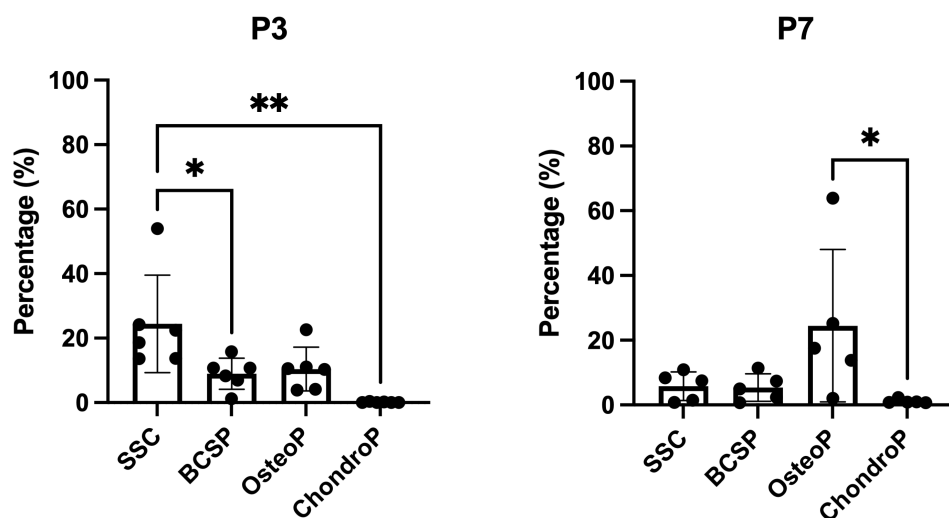


Figure 13-5 proportions of individual populations present at Passage (P) 3 and 7

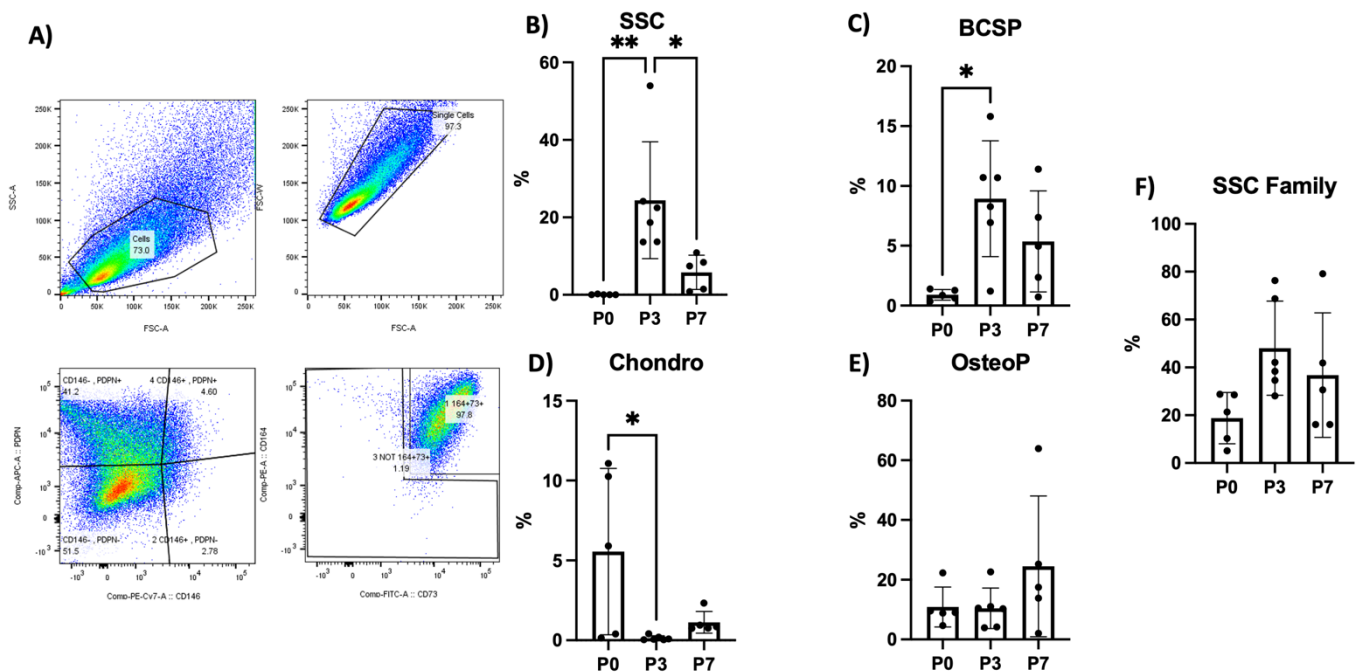


Figure 13-6 trends in individual populations over serial expansion. A) representative flow cytometry plots of cultured cells. (B – F) individual populations across passages.

13.4.5 The SSC, and associated progeny, do not maintain their cell surface marker expression during expansion

At P3, plastic adherent stromal cells were sorted and expanded as 4 groups, namely the SSC, BCSP, OsteoP and the unsorted MSC. The ChondroP was not expanded due to low cell number yields following cell sorting. At P7, each subgroup underwent flow cytometry to assess the relative expression following serial expansion. Most notably, a considerable portion of all cell populations lost expression of all 4 relevant surface markers across all groups. For the SSC subgroup, at P7 13.04% (+/- 8.478%) of cells were SSC, 5.497% (+/- 4.349%) were BCSP, 11.21% (+/- 8.772%) were OsteoP, 3.941% (+/- 2.947%) were ChondroP and 66.32% (+/- 15.99%) of cells were cell surface marker expression negative. For the BCSP subgroup, at P7 5.09% (+/- 3.772%) of cells were SSC, 6.38% (+/- 3.876%) were BCSP, 33.36% (+/- 19.59%) were OsteoP, 1.108% (+/-

0.978%) were ChondroP and 54.03% (+/- 18.85%) of cells were cell surface marker expression negative. For the OsteoP subgroup, at P7 0.853% (+/- 1.12%) of cells were SSC, 1.618% (+/- 0.948%) were BCSP, 46.61% (+/- 30.63%) were OsteoP, 0.11% (+/- 0.097%) were ChondroP and 50.81% (+/- 30.12%) of cells were cell surface marker expression negative. For the MSC subgroup, at P7 5.789% (+/- 4.43%) of cells were SSC, 5.362% (+/- 4.222%) were BCSP, 24.49% (+/- 23.56%) were OsteoP, 1.122% (+/- 0.679%) were ChondroP and 63.24% (+/- 26.03%) of cells were cell surface marker expression negative. Figure 13-7

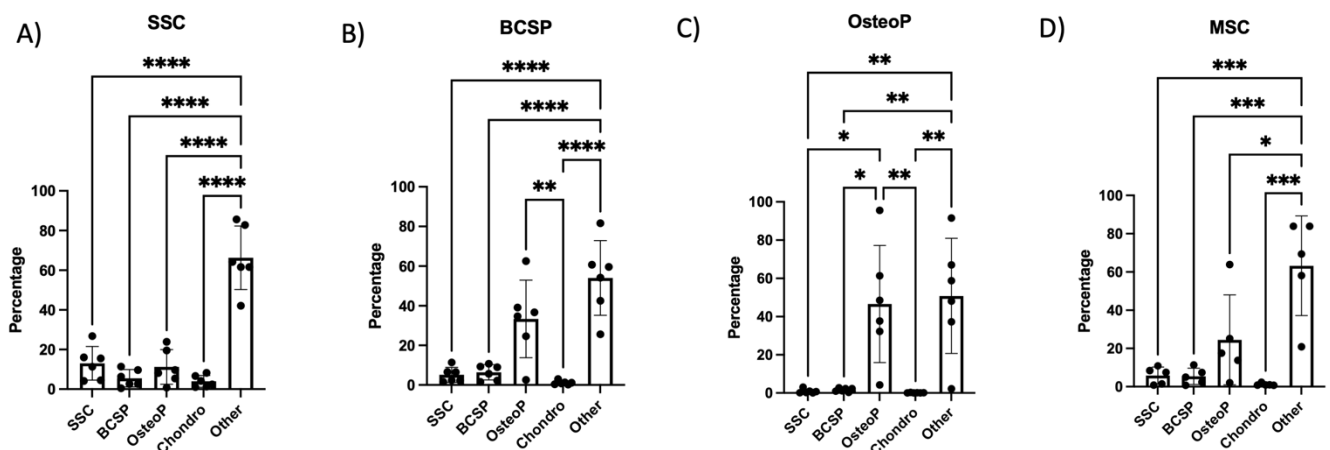


Figure 13-7 relative expression of each subpopulation following flow cytometric analysis of cultured A) SSC B) BCSP C) OsteoP D) MSC at P7

13.5 Discussion

The identification of the hSSC marked a potentially revolutionary discovery in the field of bone regeneration and tissue engineering. In recent years, experts in the scientific and clinical literature have called for more stringent definitions of stem cells, in an effort to quell the lumping together of widely diverse and heterogeneous populations under the umbrella “mesenchymal stem cell” term. The hSSC, with stem-like properties and a defined cell surface marker expression, offers such a solution. However, despite considerable interest in this cell, as evidenced by more than 330 citations on the seminal paper in the 4 years since its publication, very few groups have sought to investigate the potential of this cell in the human population. Ambrosi et al⁴⁰⁹ reported on hSSCs harvested from 5 different human skeletal sites, namely ankle, radius / ulna, clavicle, acetabulum and tibial plateau. In this study, we successfully isolated the hSSC from human vertebral bone marrow from patients undergoing instrumented spinal fusion. To our knowledge, this is the first study to report on hSSCs from the human spine or vertebra. Given the differing embryological origins of the vertebrae versus the appendicular skeleton, with the vertebrae arising from the paraxial mesoderm⁴¹² and the appendicular skeleton arising from the lateral plate mesoderm⁴¹³, an assumption that the hSSC would be present in vertebrae was not possible.

A number of clinical studies have compared the use of bone marrow aspirate (BMA), a source of progenitor cells, to the gold standard ICBG with promising results. Neen et al reported on a BMA combination with Healos (a Type 1 collagen / hydroxyapatite matrix) to be non-inferior to ICBG in posterolateral spinal fusion, but radiologically inferior in lumbar interbody fusion²¹². Kitchel et al found equivalent radiographic results in patients undergoing posterolateral fusion with one side augmented with ICBG and the other side with a mineralised collagen scaffold mixed with BMA²⁰⁷. Vaccaro et al, in a 54 patient trial, reported equivalent outcomes in patients with DBM combined with BMA versus ICBG²⁰⁴. In order to maximise the purity of cells applied to these scaffolds, a number of studies compared culture expanded MSCs to ICBG in spinal fusion. De Frutos et al conducted a randomised trial comparing human cancellous bone tissue that had been seeded with MSCs cultured ex vivo for 21 days prior to the fusion procedure²²⁸. Here, patients in the experimental arm underwent bone marrow aspiration

from the posterior iliac crest 21 days preoperatively in order to obtain the biological product. Overall, patients in the experimental arm demonstrated superior radiographic results compared to the ICBG but not in terms of patient reported outcome measures. In each of these studies, the BMA had been obtained from the iliac crest. However, the ability to harvest or utilise cells from the bone marrow of vertebrae presents an exciting proposition to spinal surgeons and has been the subject of much work over the last number of decades. This is in part due to the regular requirement to supplement the spinal fusion site with bone graft, or similar, to ensure satisfactory fusion. The ability to use locally sourced tissue negates the need to harvest from other sites, thus reducing operative time and morbidity from the additional surgical site. McLain et al⁴¹⁴ and Risbud et al³⁹⁴ compared the qualities of bone marrow harvested from vertebral bodies to iliac crest and reported higher concentrations of progenitor populations within vertebrae. Min et al⁴¹⁵ described superior in vitro osteogenic and proliferative capacity of MSCs harvested from VB compared to IC. These studies serve to confirm the suitability of the VB as a candidate to supply bone marrow progenitor cells for use in spinal surgery.

In general, it is expected that approximately 0.001 – 0.01% of non-hematopoietic, multipotent cells in a BMA consist of MSCs⁴¹⁶. We demonstrated the presence of the hSSC to be 0.054% (+/- 0.099%), within a vertebral body bone marrow aspirate following exclusion of hematopoietic cells. While a favourable result, care must be taken in their interpretation as this value was recorded following chemical digestion of the BMA which may skew cell count proportions. However, it would appear that the hSSC occurs with a relatively similar, low frequency to the traditional MSC, consistent with the expectant frequency of progenitor cells in a BMA. The use of concentrated bone marrow aspirate (cBMA) has been trialled to increase the proportion of progenitor cells in a fresh aspirate with favourable results⁴¹⁷. However, with such low cell proportions, it is clear that it is not feasible to attempt to isolate a single, pure, progenitor population, such as the hSSC, and use intraoperatively in a single sitting. Therefore, culture expanded cells, such as those used by De Frutos et al²²⁸, offer a desirable option for those wishing to deliver a pure population of cells. We report that the relative proportions of each of the hSSC lineage was greater than the fresh BMA following plastic-adherent cell culture expansion and offers a potential option for those wishing to isolate the hSSC. However, following cell sorting and individual cell culture expansion, these cells failed to maintain their cell surface marker expression over serial expansion. Therefore, if the intention is to isolate a pure population of these cells, culture following cell sorting is likely to dilute the

proportion of hSSCs within the cultured cells and the use of freshly sorted cells is the best method to deliver a pure population of hSSCs.

This study has several limitations. Firstly, the low number of patients recruited limits generalisability. Furthermore, our cohort has a broad age range and as age is known to affect MSC performance, it may potentially affect hSSC cell surface marker expression. Future work with larger numbers will be required to offset this limitation. Moreover, plastic adherent culture may affect surface marker expression, and therefore skew the results of the cell sorting and flow cytometry results. Stringent in-vivo validation of cultured cells to demonstrate stem-ness would therefore be required, prior to be considered for clinical application.

In conclusion, we have explored the potential utility of the hSSC in the spinal surgical setting. We have demonstrated a low observed frequency of hSSCs within a marrow aspirate, therefore necessitating the need for culture expanded cells if cell-based cell therapy is to be considered. Furthermore, we trialled sorting and culture expanding the hSSC from a pool of MSCs and established that the cells lose their surface marker expression over serial expansion.

Chapter 14 Discussion

14.1 Overview of Spinal Fusion Challenges

Spinal fusion surgery has become an essential procedure for treating a wide array of spinal pathologies, from trauma to degenerative diseases. While the introduction of novel techniques such as robotic-assisted surgeries, advanced imaging, and improved graft materials has enhanced outcomes, significant challenges remain, particularly in older and frail populations. The demographic shift towards an aging population presents an increasing burden on spinal surgery, with conditions like osteoporosis and frailty compounding the risk of poor surgical outcomes. In this context, enhancing bone healing in spinal fusion is of paramount importance. This thesis seeks to address these challenges by investigating the role of human skeletal stem cells (hSSCs) in spinal fusion, alongside evaluating traditional and novel graft techniques.

14.2 Advances in Technology and their Limitations

The thesis highlights significant technological advancements in spinal fusion, particularly the use of robotic-assisted surgery and computer-guided navigation. These technologies have greatly improved precision in pedicle screw placement, reduced the incidence of complications, and allowed for more complex spinal surgeries, even in patients previously considered too frail. Despite these advances, the data suggest that while these innovations enhance surgical accuracy, they do not always directly translate into improved patient outcomes, particularly in terms of fusion success rates.

The discussion on imaging techniques, such as the use of Hounsfield units (HU) to diagnose osteoporosis, further illustrates the complexity of managing age-related conditions. The meta-analysis conducted in this thesis demonstrates that HUs can be a useful diagnostic tool, but variability in thresholds and limited sensitivity in certain cases mean that no single method currently provides a definitive assessment of bone quality preoperatively.

14.3 Skeletal Stem Cells: A Promising Future?

The potential of human skeletal stem cells (hSSCs) represents a major breakthrough in regenerative medicine and tissue engineering. Unlike mesenchymal stromal cells (MSCs), which have been used with mixed success in spinal fusion, hSSCs exhibit more defined and targeted differentiation capabilities, particularly in promoting bone and cartilage formation. The discovery of these cells has the potential to revolutionize the approach to bone healing, offering a more homogeneous and potent cellular therapy for spinal fusion.

This thesis emphasizes that hSSCs, with their ability to self-renew and differentiate into osteogenic and chondrogenic lineages, could mitigate some of the shortcomings associated with MSC-based therapies, such as inconsistent cell populations and a lack of purity. By utilizing hSSCs in spinal fusion, particularly for patients with osteoporotic bone or poor healing capacity, the potential for more predictable and successful fusion outcomes could increase significantly.

14.4 Comparison of Graft Techniques

The systematic reviews and meta-analyses comparing iliac crest bone graft (ICBG) and cell-based grafts to augment spinal fusion reveal important insights. ICBG remains the gold standard in many settings due to its osteoconductive, osteoinductive, and osteogenic properties. However, limitations such as donor site morbidity and the need for large graft volumes highlight the need for alternatives. Cell-based therapies, particularly those using MSCs, have shown promise, but their inconsistent efficacy underscores the need for further refinement.

Similarly, the exploration of recombinant human bone morphogenetic proteins (rhBMP) presents both opportunities and risks. While rhBMPs have demonstrated the ability to enhance fusion rates, concerns over complications, including ectopic bone formation and potential tumorigenesis, temper their widespread adoption. The findings of this thesis suggest that while rhBMP remains a powerful tool in certain cases, careful consideration of patient factors and long-term safety is essential.

14.5 Personalized Treatment and Future Directions

The intersection of technology, biology, and surgical innovation presents a future in which spinal fusion can become more personalized. The potential to stratify patients based on preoperative bone quality (assessed via advanced imaging) and to tailor graft materials, including the use of hSSCs, represents a paradigm shift in spinal surgery. This personalized approach can reduce the rates of pseudarthrosis and improve functional outcomes, particularly in older and more vulnerable patients.

Looking forward, integrating these findings into clinical practice will require further translational research. The optimization of hSSC harvesting, expansion, and delivery is necessary to ensure consistent results. Moreover, long-term clinical trials comparing traditional techniques with cell-based therapies are essential to validate the efficacy of hSSCs in improving spinal fusion outcomes.

14.6 Limitations of the Current Research

Despite the promising findings, there are limitations that must be acknowledged. First, the heterogeneity of the patient populations in the systematic reviews and meta-analyses, particularly with regard to age, comorbidities, and preoperative bone quality, complicates direct comparisons between graft techniques. Furthermore, the research on hSSCs is still in its early stages, and while preliminary results are encouraging, the clinical application of these cells requires further investigation to confirm their safety and efficacy.

Additionally, the thesis highlights the need for standardized diagnostic criteria, particularly for conditions like osteoporosis and frailty, to ensure that preoperative assessments are accurate and reproducible across clinical settings.

14.7 Conclusion

This thesis offers a comprehensive exploration of the challenges and advancements in spinal fusion, with a particular focus on the potential of hSSCs to revolutionize treatment. The findings suggest that the integration of new biological therapies, combined with

advanced surgical techniques, holds significant promise for improving outcomes in spinal fusion, particularly in the aging population. However, achieving these results will require continued research into the optimization of graft materials, the refinement of surgical techniques, and the long-term safety of new biological therapies.

As the field continues to evolve, the work presented in this thesis provides a foundation for future studies that will shape the next generation of spinal fusion techniques, ultimately improving patient care and outcomes.

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