ISSN 2218-5836 (online)

World Journal of *Orthopedics*

World J Orthop 2019 April 18; 10(4): 176-218

Published by Baishideng Publishing Group Inc

World Journal of Orthopedics

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Monthly Volume 10 Number 4 April 18, 2019

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We encourage authors to submit their manuscripts to *WJO*.

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RESPONSIBLE EDITORS FOR THIS ISSUE

Responsible Electronic Editor: *Yun-Xiaojian Wu* **Proofing Editorial Office Director:** *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Orthopedics

ISSN
ISSN 2218-5836 (online)

LAUNCH DATE
November 18, 2010

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Bao-Gan Peng

EDITORIAL BOARD MEMBERS
http://www.wjgnet.com/2218-5836/editorialboard.htm

EDITORIAL OFFICE
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PUBLICATION DATE
April 18, 2019

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World J Orthop 2019 April 18; 10(4): 176-191

DOI: 10.5312/wjo.v10.i4.176

ISSN 2218-5836 (online)

SYSTEMATIC REVIEW

Investigational growth factors utilized in animal models of spinal fusion: Systematic review

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Abstract

BACKGROUND

Over 400000 Americans annually undergo spinal fusion surgeries, yet up to 40% of these procedures result in pseudoarthrosis even with iliac crest autograft, the current "gold standard" treatment. Tissue engineering has the potential to solve this problem *via* the creation of bone grafts involving bone-promoting growth factors (*e.g.*, bone morphogenetic protein 2). A broad assessment of experimental growth factors is important to inform future work and clinical potential in this area. To date, however, no study has systematically reviewed the investigational growth factors utilized in preclinical animal models of spinal fusion.

AIM

To review all published studies assessing investigational growth factors for spinal fusion in animal models and identify promising agents for translation.

METHODS

We conducted a systematic review of the literature using PubMed, Embase, Cochrane Library, and Web of Science databases with searches run on May 29th, 2018. The search query was designed to include all non-human, preclinical animal models of spinal fusion reported in the literature without a timespan limit. Extracted data for each model included surgical approach, level of fusion, animal species and breed, animal age and sex, and any other relevant characteristics. The dosages/sizes of all implant materials, spinal fusion rates, and follow-up time points were recorded. The data were analyzed and the results reported in tables and text. PRISMA guidelines were followed for this systematic review.

Conflict-of-interest statement: Goodwin CR: Grants from NIH/NINDS K12 Physician Scientist Award, North Carolina Spine Society, and Burroughs Wellcome Funds; all outside the submitted work. Lo SF: Research support from Chordoma Foundation, Grant from AO Spine; all outside the submitted work. Sciubba DM: Consulting from Baxter, DePuy Synthes, Globus, K2M, Medical Device Business Services, Medtronic, NuVasive, and Stryker; Speaking/Teaching from Globus Medical, Medtronic, and DePuy Synthes; all outside the submitted work. Theodore N: Royalties from Globus Medical, Depuy Synthes; Stock in Globus Medical; Consulting from Globus Medical; Scientific Advisory Board from Globus Medical; Fellowship Support from AO North America; all outside the submitted work. Witham TF: Grants from Eli Lilly Company and the Gordon and Marilyn Macklin Foundation.

Manuscript source: Unsolicited manuscript

Received: October 22, 2018
Peer-review started: October 23, 2018
First decision: November 15, 2018
Revised: January 3, 2019
Accepted: January 26, 2019
Article in press: January 26, 2019
Published online: April 18, 2019

P-Reviewer: Pavone V; Paschos NK; Guerado E
S-Editor: Dou Y
L-Editor: A
E-Editor: Wu YXJ

RESULTS

Twenty-six articles were included in this study, comprising 14 experimental growth factors: AB204 (n = 1); angiopoietin 1 (n = 1); calcitonin (n = 3); erythropoietin (n = 1); basic fibroblast growth factor (n = 1); growth differentiation factor 5 (n = 4), combined insulin-like growth factor 1 + transforming growth factor beta (n = 4); insulin (n = 1); NELL-1 (n = 5); noggin (n = 1); P-15 (n = 1); peptide B2A (n = 2); and secreted phosphoprotein 24 (n = 1). The fusion rates of the current gold standard treatment (autologous iliac crest bone graft, ICBG) and the leading clinically used growth factor (BMP-2) ranged widely in the included studies, from 0-100% for ICBG and from 13%-100% for BMP-2. Among the identified growth factors, calcitonin, GDF-5, NELL-1, and P-15 resulted in fusion rates of 100% in some cases. In addition, six growth factors - AB204, angiopoietin 1, GDF-5, insulin, NELL-1, and peptide B2A - resulted in significantly enhanced fusion rates compared to ICBG, BMP-2, or other internal control in some studies. Large heterogeneity in animal species, fusion method, and experimental groups and time points was observed across the included studies, limiting the direct comparison of the growth factors identified herein.

CONCLUSION

Several promising investigational growth factors for spinal fusion have been identified herein; directly comparing the fusion efficacy and safety of these agents may inform clinical translation.

Key words: Spinal fusion; Growth factor; Pseudoarthrosis; Systematic review

Core tip: This is the first study to systematically review all the published investigational growth factors utilized in preclinical animal models of spinal fusion. Among the identified growth factors, calcitonin, GDF-5, NELL-1, and P-15 resulted in fusion rates of 100% in some studies. In addition, six growth factors - AB204, angiopoietin 1, GDF-5, insulin, NELL-1, and peptide B2A - resulted in significantly enhanced fusion rates compared to autologous iliac crest bone graft, BMP-2, or other internal controls in some cases. Directly comparing the fusion efficacy and safety of these growth factors may inform the development of clinically translatable materials for spinal fusion.

Citation: Cottrill E, Ahmed AK, Lessing N, Pennington Z, Ishida W, Perdomo-Pantoja A, Lo SF, Howell E, Holmes C, Goodwin CR, Theodore N, Sciubba DM, Witham TF. Investigational growth factors utilized in animal models of spinal fusion: Systematic review. *World J Orthop* 2019; 10(4): 176-191
URL: https://www.wjgnet.com/2218-5836/full/v10/i4/176.htm
DOI: https://dx.doi.org/10.5312/wjo.v10.i4.176

INTRODUCTION

Over 400000 Americans undergo spinal fusion surgeries each year, with the number increasing yearly alongside a growing and aging population[1-3]. However, pseudoarthrosis, or failed fusion, rates are reported to be as high as 40% in primary spinal fusion surgery and up to 60% in revision cases, even when the "gold standard" treatment of grafting bone from the patient's own iliac crest is used[4,5]. When this happens, patients often suffer from significant pain and disability, and remaining treatment options are limited.

Tissue engineering has the potential to solve the problem of pseudoarthrosis by promoting site-specific de novo bone generation. Classically, tissue engineering involves using a scaffold, cells, and growth factors to generate living tissues. At present, the only tissue engineered product involving a growth factor that is FDA-approved for spinal fusion is a collagen sponge delivered with recombinant human bone morphogenetic protein 2 (rhBMP-2) (INFUSE Bone Graft, Medtronic). However, this product is associated with significant complications[6,7], which are thought to arise from the supraphysiologic therapeutic dose of rhBMP-2 required for effective bone

formation[8]. In addition to rhBMP-2, recombinant human parathyroid hormone (rhPTH) and recombinant human BMP-7 (rhBMP-7) have been studied clinically in spinal fusion[9-11].

Given the potential impact of tissue engineering to advance spinal fusion, many biomaterials and bioactive agents have been investigated. However, no study to date has systematically reviewed the experimental growth factors investigated for spinal fusion in preclinical animal models. Considering the efficacy and widespread use of recombinant growth factors (*i.e.*, rhBMP-2 and rhPTH) to optimize spinal fusion, a broad assessment of experimental growth factors is essential to inform future work and clinical potential in this area[9,12]. The present study aims to systematically review all published translational animal models assessing investigational growth factors for spinal fusion and identify promising agents for translation.

MATERIALS AND METHODS

Electronic literature search

A systematic review of the literature using PubMed, Embase, Cochrane Library, and Web of Science databases was performed with searches run on May 29th, 2018, along with a review of the bibliographies of the examined articles. The search query was designed to include all non-human, preclinical animal spinal fusion models reported in the literature without a timespan limit (Table 1). PRISMA guidelines were followed for this systematic review[13].

Inclusion/Exclusion criteria

Inclusion criteria were original studies involving the implantation/administration of one or more identifiable, quantifiable, experimental growth factors (*i.e.*, not BMPs or PTH) in an animal model of spinal fusion in the English language. Growth factors were defined as peptide-based molecules that function to regulate cell division/survival.

Our exclusion criteria were studies that involved the implantation of (1) scaffolds without growth factors; (2) BMPs or PTH; (3) non-peptide-based agents; and (4) cells, platelet-rich plasma, or other processed blood products that could confound effects of the growth factors.

All potentially eligible studies meeting the inclusion criteria were determined by 2 reviewers (Cottrill E and Lessing N). A third reviewer (Ahmed AK) served as a referee, resolving any discrepancies. Articles that met predetermined criteria for exclusion were not included in the study.

Data extraction

Extracted data for each animal model included surgical approach (*e.g.*, posterolateral or anterior), level of fusion, animal species and breed, animal age and sex, and any other relevant characteristics of the animals (*e.g.*, ovariectomized or genetically mutated). For each animal group studied, dosages/sizes of all implant materials, including growth factor and scaffold, were recorded. Spinal fusion rates as assessed by manual palpation, the "gold standard" technique[14,15], or alternatively other methods (*e.g.*, micro- computed tomography (CT), plain radiographs, and histological analysis), were extracted, along with the associated follow-up time points.

RESULTS

The literature search identified 4806 total articles. Following the predetermined exclusion criteria, 26 articles assessing experimental growth factors in vertebrate animal models of spinal fusion were included in this review (Figure 1). Among the included studies, 14 experimental growth factors have been described: AB204 (n = 1); angiopoietin 1 (n = 1); calcitonin (n = 3); erythropoietin (EPO) (n = 1); basic fibroblast growth factor (bFGF) (n = 1); growth differentiation factor 5 (GDF-5) (n = 4), combined insulin-like growth factor 1 (IGF-1) + transforming growth factor beta (TGF- β) (n = 4); insulin (n = 1); NELL-1 (n = 5); noggin (n = 1); P-15 (n = 1); peptide B2A (n = 2); and secreted phosphoprotein 24 (SPP24) (n = 1). Descriptions of the growth factors are provided in Table 2[16-31]. The demographic characteristics for all included studies are provided in Table 3, and the rates of spinal fusion for all experimental groups are summarized in Table 4. Detailed information of each study is provided in Supplemental Table 1. All the included studies were preclinical animal studies (level of evidence of V).

Table 1 Search terms across 4 databases to identify experimental growth factors in animal models of spinal fusion

Databases	Search terms
PubMed (1693) Embase (1709) Cochrane Library (52) Web of Science (1352) Total Results (4806)	(spinal fusion [mesh] OR spine fusion*[tw] OR spinal fusion*[tw] OR "spondylosyndesis"[tw]) AND (animals [mesh:noexp] OR "chordata"[mesh:noexp] OR ("vertebrates"[mesh] NOT "humans"[mesh]) OR "animals, domestic"[mesh] OR "animals, exotic"[mesh] OR "animals, genetically modified"[mesh] OR "animals, laboratory"[mesh] OR "animals, outbred strains"[mesh] OR "animals, wild"[mesh] OR "animals, zoo"[mesh] OR "mice"[tw] OR "mouse" [tw] OR murine*[tw] OR "rat"[tw] OR "rats"[tw] OR rabbit*[tw] OR "leporine"[tw] OR ovine*[tw] OR sheep*[tw] OR goat*[tw] OR "caprine"[tw] OR "porcine"[tw] OR "pig" [tw] OR "pigs"[tw] OR "swine"[tw] OR "cow"[tw] OR "cows"[tw] OR "bovine"[tw] OR "horse"[tw] OR "horses"[tw] OR equine*[tw] OR "canine"[tw] OR "feline"[tw] OR "animal"[tw] OR "animals" [tw] OR "dog"[tw] OR "dogs"[tw] OR "cat"[tw] OR "cats"[tw] OR monkey*[tw] OR "non human primate"[tw] OR "non human primates"[tw] OR "simian"[tw] OR "ape"[tw] OR "apes"[tw] OR "gorilla"[tw] OR "gorillas"[tw] OR "piscine"[tw] OR "fish"[tw] OR "fishes"[tw] OR "goose"[tw] OR "geese"[tw] OR "fowl"[tw] OR "poultry"[tw] OR "chicken"[tw] OR "chickens"[tw])

AB204

Zheng *et al*[32] compared the fusion rates between AB204 and rhBMP-2 in a beagle posterolateral lumbar (L1-L2 and L4-L5) model. Investigating biphasic calcium phosphate (BCP), rhBMP-2 + BCP, and AB204 + BCP, they reported that the AB204 group showed a significantly higher fusion rate (90%) compared to the rhBMP-2 group (15%) and the BCP-only group (6.3%) as assessed by manual palpation at 8 wk postoperatively.

Angiopoietin 1

Park *et al*[33] investigated the effects of COMP-Ang-1 on spinal fusion in a Sprague-Dawley rat posterolateral lumbar (L3-L5) model. Investigating iliac bone allograft (Allo), bovine serum albumin (BSA)-impregnated absorbable collagen sponge + Allo, and COMP-Ang-1-impregnated absorbable collagen sponge + Allo, they reported that the COMP-Ang-1 group showed a significantly higher fusion rate (89.5%) compared to the BSA group (42.1%) and the Allo-only group (38.9%) as assessed by manual palpation at 6 wk postoperatively.

Calcitonin

Babat *et al*[34] investigated the effects of calcitonin (postoperatively) and pamidronate (pre- and postoperatively) on spinal fusion in a New Zealand White rabbit posterolateral lumbar (L5-L6) model. Fusion rates were determined for each treatment group: autologous iliac crest bone graft (autograft) alone (56%), autograft + calcitonin (68%), and autograft + pamidronate (37%). Fisher exact test showed no significant differences between groups at 5 wk postoperatively.

In addition, Liu *et al*[35] investigated the effect of daily post-operative calcitonin administration on spinal fusion in a New Zealand White rabbit posterolateral lumbar (L4-5, without wire fixation of the spinous processes; and L6-L7, with wire fixation of the spinous processes) model. With both fixation and without fixation, the bone grafts receiving calcitonin had a higher fusion rate (100% *vs* 75%), higher histological score, and increased expression of pro-osteogenic and pro-angiogenic genes [*i.e.*, Col I and BMP-2, IGF-1 and vascular endothelial growth factor (VEGF)] at 8 wk postoperatively.

Additionally, Liu *et al*[36] investigated the effects of calcitonin (postoperatively) on spinal fusion in an ovariectomized/normal Sprague–Dawley posterolateral lumbar (L4-5, with wire fixation of spinous processes) model. They found significantly enhanced fusion mass, bone mineral density, and microstructural parameters in calcitonin-treated ovariectomized animals at 12 wk postoperatively compared to non-treated ovariectomized animals as assessed *via* radiographs, micro-CT, and histologic analysis. Fusion rate was not reported.

EPO

Rolfing *et al*[37] investigated the effects of EPO (daily subcutaneous injection) on spinal fusion in a New Zealand White rabbit posterolateral lumbar (L5-L6) fusion model. At 6 wk postoperatively, the fusion rate was 86% in the EPO treated group + autograft and 71% in the autograft-alone group as assessed *via* manual palpation. Additionally, the bone fusion volume (micro-CT) and angiogenesis (actin stained blood vessels)

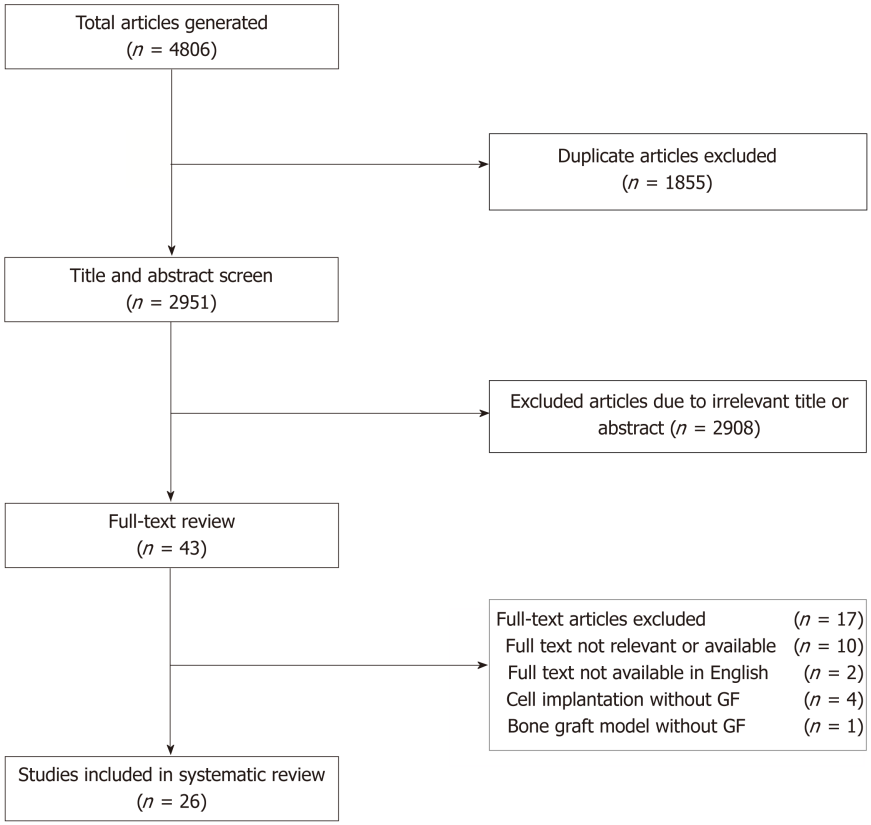

Figure 1 Consolidated standards of reporting trials diagram for article selection. GF: Growth factor.

were both significantly greater in the EPO treatment group.

bFGF

Inoue *et al*[38] investigated the effects of an engineered bFGF on spinal fusion in a Sprague-Dawley rat posterolateral lumbar (L4-L5) model. Two fusion groups were studied: Femoral freeze-dried bone allograft incubated with an engineered bFGF or with phosphate buffered saline. They found that the bFGF group had a significantly higher mean grafted bone volume (radiography) as well as significantly greater new bone formation on the surface of the laminae and spinous processes (micro-CT) compared to the control group 14 d postoperatively. Fusion rate was not reported.

GDF-5

Spiro *et al*[39] investigated the effects of rh-GDF-5 on spinal fusion in a female baboon posterolateral lumbar (L4-L5) model. They investigated four groups: Healos® with or without rh-GDF-5 or iliac crest autograft. The fusion rate, as assessed *via* radiographic/CT analysis, was 44% for the 500 micrograms rh-GDF-5/cm^3 Healos® group, 11% for the 1500 micrograms rh-GDF-5/cm^3 Healos® group, 22% for the autograft group, and 0% for the Healos® alone group at 20 wk postoperatively.

In addition, Spiro *et al*[40] investigated the effects of rh-GDF-5 in several different formulations with collagen matrices in a New Zealand White rabbit posterolateral lumbar (L5-L6) model. They found that rh-GDF-5 added to non-crosslinked mineralized Type I bovine collagen strips resulted in a fusion rate, as assessed histologically, of 75% at a concentration of 0.1 mg growth factor/cm^3 collagen and 80% at a concentration of 1.0 mg growth factor/cm^3 collagen at 12 wk postoperatively. This compared to 33% for iliac crest autograft, 0% for the collagen strips alone, and 0% for the collagen strips with bone marrow aspirate from the iliac crest.

Additionally, Jahng *et al*[41] investigated the effects of Healos® with rh-GDF-5 in a sheep endoscopic instrumented (pedicle screws and plate) posterolateral lumbar (L4-L5) model. They found that at 4 and 6 mo 100% fusion was observed in both autograft and bone graft substitute groups, and no significant differences were observed between the groups *via* histological assessment, including the formation of vascular elements.

Magit *et al*[42] investigated the effects of rh-GDF-5 in New Zealand White rabbit

Table 2 Normal biological activity of each included growth factor

Growth factors	Normal biologic activity[16-31]
AB204	Chimera of activin A and BMP-2 - which are both members of the transforming growth factor-beta (TGF-beta) superfamily
Ang-1	Pro-angiogenic growth factor that mediates reciprocal interactions between the endothelium and surrounding matrix, inhibits endothelial permeability, and contributes to blood vessel maturation and stability
Calcitonin	Secreted by the parafollicular (C cells) of the thyroid gland and is a direct inhibitor of osteoclasts
EPO	Produced in the kidney in response to hypoxia and is a well-known growth factor essential for hematopoiesis
bFGF	Broad mitogenic and angiogenic functions and is important for limb and nervous system development, wound healing, and tumor growth
GDF-5	Structurally similar to BMP-2 and BMP-7, GDF-5 is a secreted member of the TGF-beta superfamily of proteins involved in the development of various tissues and cell types, as well as the growth of neuronal axons and dendrites
IGF-1/TGF-beta	IGF-1 is a protein with similar structure and function to insulin involved in mediating growth and development. TGF-beta 3 is a secreted ligand capable of binding to various TGF-beta receptors involved in embryogenesis and cell differentiation and may play a role in wound healing
Insulin	A product of post-translational modification of proinsulin, involved in intracellular glucose uptake
NELL-1	Secreted protein containing epidermal growth factor-like repeats. It binds to the cell surface heterodimer integrin α3β1, resulting in intracellular changes that induce osteoblastogenic programming. Its overexpression is associated with craniosynostosis
Noggin	Secreted polypeptide which binds and inactivates members of the transforming growth factor-beta superfamily of proteins. Noggin is important for developmental process such neural tube closure and joint formation, and when mutated can lead to proximal symphalangism and multiple synostoses syndrome
P-15	Synthetic 15-amino acid peptide with an identical sequence to the cell-binding domain found on the α1(I) chain of Type-I collagen. It is combined with an anorganic bovine-derived hydroxyapatite matrix (ABM) to produce an osteoinductive and osteoconductive bone graft alternative
Peptide B2A	Synthetic, receptor-targeted peptide that cooperatively enhances biologic BMP-2 response
SPP24	Secreted bone matrix protein that belongs to the cystatin superfamily and binds proteins in the transforming growth factor-beta family of cytokines

BMP: Bone morphogenetic protein; GDF: Growth differentiation factor; TGF: Transforming growth factor; IGF: Insulin-like growth factor.

posterolateral lumbar (L5-L6) model. The authors found that fusion rates, as assessed via manual palpation, were 38% in the autograft group, 0% in the Healos® alone group, and 100% in each of the experimental Healos®/rhGDF-5 groups at 8 wk of follow-up. Further, *via* micro-CT analysis, bone formation in the experimental rhGDF-5 groups were observed to be significantly greater than in the other study groups.

IGF-1/TGF- β

Kandziora *et al*[43] investigated the effects of combined IGF-1/TGF-beta-1 on spinal fusion in a sheep anterolateral cervical (C3/4) interbody model. They investigated using a titanium cage alone, titanium cage filled with autologous iliac crest bone graft, titanium cage coated with a biodegradable poly-(D,L-lactide) (PDLLA) carrier including rh-BMP-2, and titanium cage coated with a biodegradable PDLLA carrier including rh-IGF-1 (5% w/w) and rh-TGF-beta-1 (1% w/w). As assessed *via* CT, the BMP-2 and IGF-1/TGF-beta-1 groups led to intervertebral masses with a maximum intervertebral gap in the craniocaudal direction of less than 5 mm or complete fusion in 75% of animals, compared to 50% for the autograft group and 25% for the cage-alone group at 12-wk postoperatively.

In addition, Kandziora *et al*[44] investigated the effects of IGF-1/TGF-beta-1 in a sheep anterolateral cervical (C3-C4) interbody model using autologous tricortical iliac crest bone graft, a titanium cage alone, titanium cage with a PDLLA carrier, and a titanium cage with a PDLLA carrier including IGF-F and TGF-beta-1. The authors observed a fusion rate of 0% in the autograft, cage-alone, and cage plus PDLLA groups and 12.5% in the IGF-1/TGF-beta-1 group at 12 weeks postoperatively.

Additionally, Kandziora *et al*[45] investigated the effects of IGF-1/TGF-beta-1 in a

Table 3 Demographic characteristics for all included studies

Animal model	Studies (*n*)
Dog	1[32]
Rat	8[33,36,38,47-49,51,56]
Rabbit	7[34,35,37,40,42,52,54]
Macaque	1[20]
Sheep	7[41,43-45,50,53,55]
Baboon	1[39]
Goat	1[46]
Spinal levels fused	
C3 – C4	4[43-46]
L1 – L2	1[32]
L2 – L3	1[55]
L3 – L4	3[20,50,56]
L4 – L5	12[32,35,36,38,39,41,47-49,51,54,55]
L3 – L5	2[33,53]
L5 – L6	7[20,34,37,40,42,50,52]
L6 – L7	1[35]
Spinal region	
Cervical	4[43-46]
Lumbar	22[20,32-42,47-56]
Biomechanical location	
Mobile segments	11[20,32,33,43-46,50,53,55,56]
Junctional segments	21[20,32-42,47-55]
Number of levels fused	
1-Level fusion	19[34,36-49,51,52,54,56]
Two separate 1-level fusions	5[20,32,35,50,55]
2-Level fusion	2[33,53]
Surgical approach	
Anterior	8[20,43-46,50,53,55]
Posterior	18[32-42,47-49,51,52,54,56]

sheep anterolateral (C3-C4) interbody model using a titanium cage coated with a PDLLA carrier including no growth factors, as well as with different concentrations of IGF-1 and TGF-beta-1. They authors concluded that the application of IGF-1 and TGF-beta-1 by a PDLLA-coated cage significantly improves interbody bone formation in a dose dependent manner, as assessed via micro-CT and histomorphometrical analysis, at 12 wk postoperatively. Fusion rate was not reported.

Further, Gu *et al*[46] investigated the effects of IGF-1 and TGF-beta-1 in a goat cervical (C3-C4) interbody model. Four groups were studied: autologous iliac crest bone graft, a hat-shaped titanium cage, a hat-shaped cage coated with hydroxyapatite, and a hat-shaped cage coated with hydroxyapatite plus IGF-1 and TGF-beta-1. As assessed *via* histomorphologic examination, the IGF-1 and TGF-beta-1 group led to fusion in 63% of animals, compared to 38% for the hydroxyapatite group, 25% for the cage alone group, and 0% for the autograft group at 12-wk postoperatively.

Insulin

Koerner *et al*[47] investigated the effects of time-released insulin on spinal fusion in a Sprague-Dawley posterolateral lumbar (L4-L5) model. The authors used iliac crest autograft plus either Linplant (95% micro-recrystallized palmitic acid and 5% bovine insulin) or a sham implant (100% palmitic acid). At 8 wk postoperatively, the fusion rate was 60% in the Linplant group compared to 11% in the control group, as assessed by manual palpation. In addition, half the animals in each group were euthanized on postoperative day 4 and analyzed for growth factors: IGF-I (but not TGF-beta-1, PDGF-AB, or VEGF) was significantly higher in the Linplant group.

Neural EGFL Like 1 (NELL-1)

Lee *et al*[48] investigated the effects of NELL-1 on spinal fusion in a male athymic rat

Table 4 Experimental models and rates of bony fusion for each included study

Growth factor	Animal(*n*)	Levels (a/p)[1]	Experimental groups	Fusion rate
AB204				
Zheng *et al*[32], 2017	Dog (*n* = 56)	L1-L2, L4-L5 (p)	(1) BCP; (2) BCP + rhBMP-2; (3) BCP + AB204	(1) 6.3%; (2) 15%; (3) 90%[2]
COMP-Ang-1				
Park *et al*[33], 2011	Rat (*n* = 56)	L3-L5 (p)	(1) ICBG; (2) ICBG + bovine serum albumin-impregnated collagen sponge; (3) ICBG + COMP-Ang-1-impregnated collagen sponge	(2) 38.9%; (2) 42.1%; (3) 89.5%[2]
Calcitonin				
Babat *et al*[34], 2005	Rabbit (*n* = 56)	L5-L6 (p)	(1) ICBG; (2) ICBG + calcitonin; (3) ICBG + pamidronate	(1) 56%; (2) 68%; (3) 37%
Liu *et al*[35], 2012	Rabbit (*n* = 32)	L4-L5, L6-L7 (p)	(1) ICBG; (2) ICBG + calcitonin; (3) ICBG + interspinous fixation (steel wire); (4) ICBG + interspinous fixation (steel wire) + calcitonin	(1) 75%; (2) 100%; (3) 75% 100%
Liu *et al*[36], 2015	Rat (*n* = 50)	L4-L5 (p)	(1) Sham surgery + saline vehicle; (2) Ovariectomy + saline vehicle; (3) Spinal fusion (ICBG) + saline vehicle; (4) Ovariectomy + Spinal fusion (ICBG); (5) Ovariectomy + Spinal fusion (ICBG) + calcitonin	Not reported
EPO				
Rolfing *et al*[37], 2011	Rabbit (*n* = 28)	L5-L6 (p)	(1) ICBG + Epoetin beta subcutaneous injection; (2) ICBG + Saline subcutaneous injection	(1) 86%; (2) 71%
bFGF				
Inoue *et al*[38], 2017	Rat (*n* = 20)	L4-L5 (p)	(1) Allograft; (2) Allograft + bFGF	Not reported
GDF-5				
Spiro *et al*[39], 2000	Baboon (*n* = 36)	L4-L5 (p)	(1) ICBG; (2) Collagen matrix strips; (3) Collagen matrix strips + rhGDF-5 (500μg/cm^3); (4) Collagen matrix strips + rhGDF-5 (1500μg/cm^3)	(1) 22%; (2) 0%; (3) 44%; (4) 11%
Spiro *et al*[40], 2001	Rabbit (*n* = 31)	L5-L6 (p)	(1) ICBG; (2) Hydroxyapatite-mineralized collagen matrix (Matrix); (3) Matrix + bone marrow; (4) Healos strips + rhGDF-5 (0.1 mg/cc); (5) Healos strips + rhGDF-5 (1.0 mg/cc); (6) Non-crosslinked collagen strips + rhGDF-5 (0.1 mg/cc); (7) Non-crosslinked collagen strips + rhGDF-5 (1.0 mg/cc); (8) Collagen fiber slurry + rhGDF-5 (0.1 mg/cc); (9) Collagen fiber slurry + rhGDF-5 (1.0 mg/cc)	(1) 33%; (2) 0%; (3) 0%; (4) 0%; (5) 67%; (6) 75%; (7) 80%; (8) 25%; (9) 0%
Jahng *et al*[41], 2004	Sheep (*n* = 8)	L4-L5 (p)	(1) ICBG; (2) Healos + GDF-5	(1)100%; (2) 100%
Magit *et al*[42], 2006	Rabbit (*n* = 65)	L5-L6 (p)	(1) ICBG; (2) Healos; (3) Healos + rhGDF-5 (0.5 mg/cc); (4) Healos + rhGDF-5 (1 mg/cc); (5) Healos + rhGDF-5 (1.5 mg/cc)	(1) 38%; (2) 0%; (3) 100%[2]; (4) 100%[2]; (5) 100%[2]
IGF-1/TGF-β				
Kandziora *et al*[43], 2002	Sheep (*n* = 32)	C3-C4 (a)	(1) Titanium cage (Cage); (2) Cage + ICBG; (3) Cage + PDLLA + BMP-2; (4) Cage + PDLLA + rh-IGF-1/TGF-β	(1) 0%; (2) 13%; (3) 13%; (4) 13%

Kandziora *et al*[44], 2002	Sheep (*n* = 32)	C3-C4 (a)	(1) ICBG; (2) Titanium cage (Cage); (3) Cage + PDLLA; (4) Cage + PDLLA + rh-IGF-1/TGF-β	(1) 0%; (2) 0%; (3) 0%; (4) 12.5%
Kandziora *et al*[45], 2003	Sheep (*n* = 32)	C3-C4 (a)	(1) Titanium cage + PDLLA (Cage); (2) Cage + rh-IGF-1/TGF-β (2.5/0.5%); (3) Cage + rh-IGF-1/TGF-β (5/1%); (4) Cage + rh-IGF-1/TGF-β (10/2%)	Not reported
Gu *et al*[46], 2006	Goat (*n* = 32)	C3-C4 (a)	(1) ICBG; (2) Titanium cage (Cage); (3) Cage + hydroxyapatite; (4) Cage + IGF-1/TGF-β (5/1%)	(1) 0%; (2) 25%; (3) 38%; (4) 63%
Insulin				
Koerner *et al*[47], 2013	Rat (*n* = 19)	L4-L5 (p)	(1) ICBG + 100% palmitic acid; (2) ICBG + Linplant (95% palmitic acid and 5% bovine insulin)	(1) 11%; (2) 60%[2]
NELL-1				
Lee *et al*[48], 2009	Rat (*n* = 10)	L4-L5 (p)	(1) Demineralized bone putty (Putty) + PBS; (2) Putty + rh-NELL-1	(1) 0%; (2) 60%
Li *et al*[49], 2010	Rat (*n* = 24)	L4-L5 (p)	(1) DBX + PBS; (2) DBX + NELL-1 (2.5 g); (3) DBX + NELL-1 (5 μg)	(1) 25%; (2) 75%; (3) 88%
Siu *et al*[50], 2011	Sheep (*n* = 32)	L3-L4 L5-L6 (a)	(1) Cage + DBM; (2) Cage + inactivated DBM; (3) Cage + DBM + NELL-1 (0.3 mg/mL) Cage + DBM + NELL-1 (0.6 mg/mL); (4) Cage + inactivated; (5) DBM + NELL-1 (0.3 mg/mL); (6) Cage + inactivated DBM + NELL-1 (0.3 mg/mL)	(1) 50%; (2) 50%; (3) 87.5%[2]; (4) 100%[2]; (5) 100%[2]; (6) 100%[2]
Yuan *et al*[51], 2013	Rat (*n* = 26)	L4-L5 (p)	(1) DBX + PBS; (2) DBX + NELL-1 (10μg); (3) DBX + NELL-1 (50μg); (4) Acellular collagen sponge (ACS) + PBS; (5) ACS + BMP-2; (6) ICBG	(1) 20%; (2) 100%; (3) 100%; (4) 0%; (5) 100%; (6) 0%
James *et al*[20], 2017	Macaque (*n* = 12)	L3-L4 L5-L6 (a)	(1) Cage + aTCP; (2) Cage + DBX + rh-NELL-1-loaded aTCP (1mg/mL); (3) Cage + DBX + rh-NELL-1-loaded aTCP (1.7mg/mL)	(1) 25%; (2) 25%; (3) 100%
Noggin				
Klineberg *et al*[52], 2014	Rabbit (*n* = 25)	L5-L6 (p)	(1) ICBG + Noggin scrambled siRNA bilateral; (2) ICBG + scrambled siRNA one side + siRNA other side; (3) ICBG + functional siRNA bilateral	(1) - (2) - (3) 50%
P-15				
Sherman *et al*[53], 2010	Sheep (*n* = 12)	L3-L5 (a)	(1) PEEK + ICBG; (2) PEEK + anorganic bovine-derived matrix/P-15	(1) 83%; (2)100%
Peptide B2A				
Smucker *et al*[54], 2008	Rabbit (*n* = 45)	L4-L5 (p)	(1) ICBG; (2) ICBG + TCP (1:1); (3) ICBG + B2A (50 μg); (4) ICBG + B2A (100 μg); (5) ICBG + B2A (300 μg)	(1) 25%; (2) 22%; (3) 56%; (4) 78%[2] (*vs* A and B); (5) 40%
Cunningham *et al*[55], 2009	Sheep (*n* = 40)	L2-L3 L4-L5 (a)	(1) PEEK + ICBG; (2) PEEK + ICBG + B2A (50 μg); (3) PEEK + ICBG + B2A (100 μg); (4) PEEK + ICBG + B2A (300 μg); (5) PEEK + ICBG + B2A (600 μg)	(1) 63%; (2) 88%; (3) 88%; (4) 88%; (5) 75%
SPP24				

Sintuu *et al*[56], 2011	Rat	(1) Collagen sponge (Sponge); (2) Sponge + rhBMP-2; (3) Sponge + rhBMP-2 (1 μg) + SPP24 (100 μg); (4) Sponge + rhBMP-2 (1 μg) + SPP24 (500 μg); (5) Sponge + rhBMP-2 (1 μg) + SPP24 (1 mg); (6) Sponge + rhBMP-2 (1 μg) + SPP24 (2.5 mg); (7) Sponge + rhBMP-2 (1 μg) + SPP18 (100 μg); (8) Sponge + rhBMP-2 (1 μg) + SPP18 (500 μg); (9) Sponge + rhBMP-2 (1 μg) + SPP18 (1 mg); (10) Sponge + rhBMP-2 (1 μg) + SPP18 (2.5 mg); (11) Sponge + rhBMP-2 (10 μg) + SPP24 (100 μg); (12) Sponge + rhBMP-2 (10 μg) + SPP24 (500 μg); (13) Sponge + rhBMP-2 (10 μg) + SPP24 (1 mg); (14) Sponge + rhBMP-2 (10 μg) + SPP24 (2.5 mg); (15) Sponge + rhBMP-2 (10 μg) + SPP18 (100 μg); (16) Sponge + rhBMP-2 (10 μg) + SPP18 (500 μg); (17) Sponge + rhBMP-2 (10 μg) + SPP18 (1 mg); (18) Sponge + rhBMP-2 (10 μg) + SPP18 (2.5 mg)	(1) 0%; (2) 100%[2]; (3) 0%; (4) 0%; (5) 0%; (6) 0%; (7) 0%; (8) 0%; (9) 0%; (10) 0%; (11) 0%; (12) 0%; (13) 0%; (14) 0%; (15) 0%; (16) 0%; (17) 0%; (18) 0%

[1](a/p): anterior/posterolateral surgical approach; N[2]: Statistically significant compared to all other experimental groups. ICBG: Iliac crest bone graft. BCP: Biphasic calcium phosphate; rhBMP-2: Recombinant human bone morphogenetic protein 2; bFGF: Basic fibroblast growth factor; GDF-5: Growth differentiation factor 5; PDLLA: Poly-D, L-lactide; TGF: Transforming growth factor; IGF: Insulin-like growth factor.

posterolateral lumbar (L4-L5) model. They found that rh-NELL-1 lyophilized onto apatite-coated alginate/chitosan microparticles and mixed with demineralized bone matrix (DBM) led to a fusion rate of 60% at 4 wk postoperatively, compared to a fusion rate of 0% using PBS instead of rh-NELL-1.

Similarly, Li *et al*[49] investigated the effects of NELL-1 on spinal fusion in an athymic rat posterolateral lumbar (L4-5) model. The authors found that NELL-1 lyophilized onto β-tricalcium phosphate (TCP) microparticles and mixed with DBX (a type of DBM) led to a fusion rate of 75% (2.5 micrograms NELL-1) and 88% (5 micrograms NELL-1) at 4 wk postoperatively, compared to a fusion rate of 25% using PBS instead of NELL-1.

Siu *et al*[50] investigated the effects of NELL-1 on spinal fusion in a skeletally mature Rambouillet × Columbian ewe posterolateral lumbar (L3-L4 and L5-L6) model. Six groups with different implant compositions were studied: DBM alone or mixed with NELL-1 (0.3 or 0.6 mg/mL) and heat-inactivated DBM (inDBM) alone or mixed with NELL-1 (0.3 or 0.6 mg/mL). At 3 mo postoperatively, the fusion rates were 88% for DBM + 0.3 mg/mL NELL-1, 100% for DBM + 0.6 mg/mL NELL-1, and 50% for DBM alone. At 4 mo postoperatively, the fusion rates were 100% for inDBM + 0.3 mg/mL NELL-1, 100% for inDBM + 0.6mg/mL NELL-1, and 50% for inDBM alone.

Yuan *et al*[51] investigated the effects of NELL-1 in a male athymic rat posterolateral lumbar (L4-5) model. They found that DBX mixed with NELL-1 (10 or 50 microgram) led to a 100% fusion rate at 4 wk postoperatively, compared to fusion rates of 20% using PBS instead of NELL-1, 100% using an acellular collagen sponge and BMP-2 (90 micrograms), and 0% using iliac crest autograft.

James *et al*[20] investigated the effects of NELL-1 on spinal fusion in a 5- to 7-year-old Rhesus macaque posterolateral lumbar (L3-L4 and L5-L6) model. Three groups were studied: intervertebral cage plus DBX mixed with saline- or rh-NELL-1-(1.0 or 1.7 mg/mL) loaded apatite-coated β-tricalcium phosphate (aTCP) particles. At 4 mo postoperatively, fusion rates as assessed *via* CT were 100% for the higher dose of rh-NELL-1, 25% for the lower dose of rh-NELL-1, and 25% for the saline control. Additionally, immunofluorescence staining showed increased Sca-1+CD31–CD45– stromal cells in the rh-NELL-1 treated groups compared to the saline control.

Noggin

Klineberg *et al*[52] investigated the effects of noggin on spinal fusion in a skeletally mature New Zealand White rabbit posterolateral lumbar (L5-L6). Noggin siRNA was injected into the paraspinal muscles to interrupt the negative feedback loop on endogenous BMP. Autologous iliac crest bone graft with paraspinal injections of

either scrambled (non-functional) siRNA bilaterally, scrambled siRNA on one side of the spine and functional noggin siRNA on the other, or functional noggin siRNA bilaterally were studied. As assessed via manual palpation, the fusion rate of the bilateral functional siRNA group was 50%, which was not significantly different compared to historical autograft-only controls from the group, despite the fact that noggin protein was successfully knocked down *in vivo* for the initial 7 days before returning to normal levels by 6 wk.

P-15

Sherman *et al*[53] investigated the effects of an organic bovine-derived hydroxyapatite matrix combined with a synthetic 15 amino acid residue (ABM/P-15) on spinal fusion in a skeletally mature ewe anterolateral lumbar (L3-L5) model. Sheep were treated with polyetheretherketone (PEEK) interbody rings filled with autologous iliac crest bone graft at one level and AMB/P-15 formulated in a carboxymethylcellulose hydrogel matrix at the other. At 6 mo postoperatively, 100% of fusion sites in both groups achieved successful bony arthrodesis as assessed *via* CT, and histomorphometric analysis showed no statistically significant differences in the fusion masses between these groups.

Peptide B2A

Smucker *et al*[54] investigated the effects of B2A on spinal fusion in a skeletally mature New Zealand White rabbit posterolateral lumbar (L4-L5) model. The authors investigated iliac crest autograft alone and 1:1 mixtures of autograft and B2A-coated ceramic granules (CG) (0, 50, 100, and 300 μg B2A/mL CG). As assessed *via* manual palpation at 6 wk postoperatively, the fusion rates were 25% for the autograft alone group and 22%, 56%, 78%, and 40% for the 0, 50, 100, and 300 μg B2A/mL CG groups, respectively. The newly formed bone in the B2A-treated groups appeared morphologically normal without hyperplasia.

Cunningham *et al*[55] investigated the effects of B2A on spinal fusion in a 3- to 6-year-old crossbred Suffolk sheep anterolateral lumbar (L2-L3 and L4-L5) model. The sheep were treated with a PEEK interbody cage packed with 1:1 mixtures of autograft and B2A-coated ceramic granules (CG) (0, 50, 300, or 600 μg B2A/mL CG). As assessed *via* CT at 4 mo postoperatively, the fusion rates were 63%, 88%, 88%, and 75% for the 0, 50, 300, and 600 μg B2A/mL CG groups, respectively. In biomechanical testing, no statistically significant differences were observed between any of the groups.

SPP24

Sintuu *et al*[56] investigated the effects of SPP24 on spinal fusion in a 6- to 8-wk-old male Lewis rat posterolateral lumbar (L3-L4) model. Bilaterally placed implant materials consisted of collagen sponges soaked in high or low dose rh-BMP-2 (1 or 10 micrograms), plus treatment: 0, 0.1, 0.5, 1.0, or 2.5 mg of either full-length SPP24 or truncated SPP18 (solely the BMP-binding region of the full peptide). As assessed *via* manual palpation at 8 wk postoperatively, the fusion rate was 0% in all specimens treated with rh-BMP-2 and TR-spp18 or FL-spp24 (any concentration), compared to a fusion rate of 100% in specimens treated with rh-BMP-2 (10 micrograms) alone. Further, FL-spp24 showed a greater inhibitory impact compared to TR-spp18.

DISCUSSION

Pseudoarthrosis following spinal surgery can lead to significant patient morbidity and diminished quality of life, with unpredictable clinical outcomes following revision surgery. Optimizing the rate of spinal fusion relies on enhanced surgical technique, effective biologics (*e.g.*, growth factors), instrumentation, and a greater appreciation of the local physiology[57]. Following FDA approval in 2002 for use in the anterior lumbar spine, rhBMP-2 revolutionized the role for growth factor adjuncts in spinal fusion[58,59], drastically increasing in use from 5.5% of all fusion cases in 2003 to 28.1% of all fusion cases in 2008 in the United States[60]. However, rhBMP-2 is associated with significant complications[6,7], which has fueled the investigation of different growth factors for spinal fusion. Despite significant research interest in this area, there are no published systematic reviews summarizing the state of the art in experimental growth factors for spinal fusion.

The present systematic review, across 4 databases, resulted in the inclusion of 26 spinal fusion animal studies comprising 14 investigational growth factors. The fusion rates of the current gold standard treatment (autologous iliac crest bone graft, ICBG) and the leading clinically used growth factor (BMP-2) ranged widely in the included studies, from 0-100% for ICBG[44,46,51] and from 13%-100% for BMP-2[43,51,56]. This

variation reflects the unpredictable clinical outcomes following spinal fusion surgery and supports the need for efficacious materials that promote strong and reliable spinal fusion. Among the experimental growth factors, four resulted in fusion rates of 100% in some cases (Table 4): calcitonin[35], GDF-5[41,42], NELL-1[20,50,51], and P-15[53] (Table 4). In addition, six growth factors resulted in significantly enhanced fusion rates compared to ICBG, BMP-2, or other internal control in some studies (Table 4): AB204 *vs* BMP-2[32], COMP-Ang-1 *vs* ICBG[33], GDF-5 *vs* ICBG[42], insulin (as Linplant) *vs* internal control (ICBG plus sham implant)[47], NELL-1 *vs* internal control (DBM)[50], and Peptide B2A *vs* ICBG[54]. The majority of other identified growth factors resulted in fusion rates similar to ICBG (Table 4); only SPP24 was shown to significantly decrease the rate of spinal fusion[56]. Directly comparing different growth factors herein is difficult given the extensive heterogeneity in animal species, fusion method, and experimental groups and timepoints across the studies (Tables 3 and 4). Further, it is known that the scaffolds themselves affect bone formation[61,62], possibly confounding the effects of the growth factors across studies.

In addition, similar effects on spinal fusion were generally, though not always, observed when multiple studies investigated the same growth factor. For example, several groups investigated the effects of NELL-1 on spinal fusion[20,48-51]. In all these experiments, NELL-1 was shown to enhance fusion rates. In contrast, for GDF-5, Magit *et al*[42] observed a significantly enhanced fusion rate compared to ICBG, while Jahng *et al*[41] observed a 100% fusion rate for both GDF-5 and ICBG groups, and Spiro *et al*[39] observed a non-significant decrease in fusion rate using GDF-5 (1500 micrograms/cm^3) compared to ICBG. The differences in animal species (rabbit, sheep, and baboon) and surgical method (endoscopic with instrumentation and posterolateral without instrumentation) may help to account for these variations.

The successful clinical translation of any factor intended to enhance spinal fusion will depend not only on its capacity to promote strong and reliable spinal fusion in humans, but also on its safety profile (*i.e.*, the associated local and systemic complications). At present, the growth factors AB204, COMP-Ang-1, GDF-5, NELL-1, P-15, insulin, and Peptide B2A represent some of the most promising investigational growth factors for promoting spinal fusion, with each demonstrating fusion efficacy in preclinical studies. However, the safety profiles of these growth factors in the setting of spinal fusion are largely unknown. In our review, none of the included studies reported complications directly related to the growth factors, though this absence of evidence obviously does not mean the absence of complications, any of which could hinder or halt clinical translation. Future work investigating the efficacy and safety of these growth factors not only in larger numbers of animals but also in higher-order species will be important for informing their potential clinical translation.

Interestingly, this systematic review found that, within the inclusion/exclusion criteria of our study, relatively few (*i.e.*, fourteen) unique growth factors have been investigated in preclinical animal models of spinal fusion. This reveals that a relatively select group of growth factors in the overall setting of bone tissue engineering has been investigated in spinal fusion. For example, growth factors like stromal-derived growth factor 1 (SDF-1) and platelet derived growth factor (PDGF), both of which have been studied in the setting of regenerating critical sized bone defects[63,64], are notably absent in the preclinical spine fusion literature. While tissue engineering for spinal fusion is unique from other areas of bone tissue engineering in that the fusion site may be in motion during the fusion process, our review suggests potential new research strategies regarding the investigation of currently unexplored growth factors (*e.g.*, SDF-1 and PDGF) for spinal fusion. Lastly, it is notable that relatively few studies involved combinations of growth factors[43-46]. We believe that the simultaneous or sequential delivery of multiple different growth factors may result in a synergistic enhancement in spinal fusion. We encourage future work in these areas, as well as in continued advancements in growth factor delivery methods and scaffold materials, towards the development of efficacious and safe, clinically translatable materials for spinal fusion.

Schimandle *et al*[65] in 1994, Sandhu *et al*[66] in 2002, and Drespe *et al*[67] in 2005 previously published reviews of animal models for spinal fusion. These reviews focused on the different species utilized, technical methodology, and representative outcomes. To the best of our knowledge, the present study is the first to systematically review investigational growth factors utilized in animal models of spinal fusion. Despite the novelty of this review, there are several limitations, including those inherent to systematic reviews. Additionally, many of the animal models differ with regard to the methodology and data collected (Tables 3 and 4). As a result of this heterogeneity, directly comparing end points (*i.e.*, rates of fusion) across multiple studies is not possible. Further, three studies did not report fusion rates[36,38,45], limiting the interpretability of those studies. In addition, our review excludes growth factors

that have been studied clinically in spinal fusion.

In conclusion, this is the first study to systematically review all the published investigational growth factors utilized in preclinical animal models for spinal fusion. Future studies aimed at directly comparing the most promising experimental growth factors identified herein - *e.g.*, AB204, COMP-Ang-1, GDF-5, NELL-1, P-15, insulin, Peptide B2A, and others (Table 4) - in preclinical models may inform the development of efficacious, clinically translatable materials for spinal fusion. Further, future work involving the safety and cost of production of these growth factors, in comparison to BMP-2, may support the replacement of BMP-2 for safer and more cost-effective growth factors for spinal fusion.

ARTICLE HIGHLIGHTS

Research background

Over 400000 Americans undergo spinal fusion surgeries each year, with the number increasing yearly alongside a growing and aging population. However, pseudoarthrosis, or failed fusion, rates are reported to be as high as 40% in primary spinal fusion surgery and up to 60% in revision cases, even when the "gold standard" treatment of grafting bone from the patient's own iliac crest is used.

Research motivation

To date, no study has systematically reviewed the experimental growth factors investigated for spinal fusion in preclinical animal models. Considering the efficacy and widespread use of recombinant growth factors (*i.e.*, rhBMP-2 and rhPTH) to optimize spinal fusion, a broad assessment of experimental growth factors is essential to inform future work and clinical potential in this area.

Research objectives

Systematically review all published translational animal models assessing investigational growth factors for spinal fusion and identify promising agents for translation.

Research methods

A systematic review of the literature using PubMed, Embase, Cochrane Library, and Web of Science databases was performed. Inclusion criteria were original studies involving the implantation/administration of one or more identifiable, quantifiable, experimental growth factors (*i.e.*, not BMPs or PTH) in an animal model of spinal fusion in the English language. Exclusion criteria were studies that involved the implantation of (1) scaffolds without growth factors; (2) BMPs or PTH, (3) non-peptide-based agents, and (4) cells, platelet-rich plasma, or other processed blood products that could confound effects of the growth factors. PRISMA guidelines were followed for this systematic review.

Research results

The literature search identified 4806 total articles, from which 26 articles met the inclusion/exclusion criteria and were included in this review. Among the included studies, 14 experimental growth factors were identified: AB204 (n = 1); angiopoietin 1 (n = 1); calcitonin (n = 3); erythropoietin (n = 1); basic fibroblast growth factor (n = 1); growth differentiation factor 5 (n = 4), combined insulin-like growth factor 1 + transforming growth factor beta (n = 4); insulin (n = 1); NELL-1 (n = 5); noggin (n = 1); P-15 (n = 1); peptide B2A (n = 2); and secreted phosphoprotein 24 (n = 1). Among the identified growth factors, calcitonin, GDF-5, NELL-1, and P-15 resulted in fusion rates of 100% in some cases. In addition, six growth factors - AB204, angiopoietin 1, GDF-5, insulin, NELL-1, and peptide B2A - resulted in significantly enhanced fusion rates compared to ICBG, BMP-2, or other internal control in some studies. Large heterogeneity in animal species, fusion method, and experimental groups and time points was observed across the included studies, limiting the direct comparison of the growth factors identified herein.

Research conclusions

This is the first study to systematically review all the published investigational growth factors utilized in preclinical animal models of spinal fusion. Future studies aimed at directly comparing the most promising investigational growth factors identified herein - *e.g.*, AB204, COMP-Ang-1, GDF-5, NELL-1, P-15, insulin, Peptide B2A, and others - in preclinical models may inform the development of efficacious and safe, clinically translatable materials for spinal fusion.

Research perspectives

The successful clinical translation of any factor intended to enhance spinal fusion will depend not only on its capacity to promote strong and reliable spinal fusion in humans, but also on its safety profile. Our study reveals that relatively few growth factors and delivery strategies in the overall setting of bone tissue engineering have been investigated in spinal fusion. We encourage future investigation of currently unexplored growth factors for spinal fusion, as well as continued advancements in growth factor delivery methods and scaffold materials, towards the development of efficacious and safe, clinically translatable materials for spinal fusion.

ACKNOWLEDGEMENTS

We would like to thank Ms. Carrie Price, MLS, for technical assistance with the literature search.

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World Journal of Orthopedics

Submit a Manuscript: https://www.f6publishing.com

World J Orthop 2019 April 18; 10(4): 192-205

DOI: 10.5312/wjo.v10.i4.192

ISSN 2218-5836 (online)

SYSTEMATIC REVIEW

Growing pains: What do we know about etiology? A systematic review

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Author contributions: Pavone V, Vescio A and Testa G contributed equally to the work; Valenti F conceptualized and designed the review together with Vescio A and Testa G carried out the analysis; Sapienza M and Sessa G drafted the initial manuscript; all authors reviewed and approved the final manuscript as submitted.

Conflict-of-interest statement: All authors declare no conflicts of interest.

PRISMA 2009 Checklist statement: A PRIMA checklist was uploaded.

Manuscript source: Unsolicited manuscript

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Abstract

BACKGROUND

Growing pains is the most common cause of musculoskeletal pain in early childhood and was first described in 1823 by French physician Marcel Duchamp. Although it has been researched extensively, the etiology is still unknown. Several theories have been proposed throughout the years.

AIM

Analyze the available scientific literature to provide an update on the latest evidence on the etiology.

METHODS

According to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses, the scientific literature on the etiology of growing pains was systematically reviewed using the following inclusion criteria: studies of any level of evidence reporting clinical or preclinical results and dealing with the etiology of growing pains. The medical electronic databases PubMed and Web of Science were searched by two independent authors on October 20, 2018. The search string used was "(growing pains OR benign nocturnal limb pains OR musculoskeletal pains) AND (etiology OR pathogenesis) AND (pediatrics)".

RESULTS

A total of 32 articles were included. The etiology of growing pains still remains poorly understood. Many theories have been proposed, but none of them are decisive. A lower pain threshold has been found among patients suffering from growing pains in comparison to healthy controls. Furthermore, evidence suggests an association between growing pains and reduced bone strength in young patients, although this finding still remains controversial. Changes in the vascular

Received: January 2, 2019
Peer-review started: January 4, 2019
First decision: January 29, 2019
Revised: February 15, 2019
Accepted: March 16, 2019
Article in press: March 16, 2019
Published online: April 18, 2019

P-Reviewer: Kaspiris A
S-Editor: Ji FF
L-Editor: A
E-Editor: Wu YXJ

perfusion pattern have also been studied. However, the etiology of growing pains does not seem related to a vascular component. The anatomical/mechanical theory has not been supported, but the role of vitamin D deficiency has been investigated many times. Strong recent evidence indicates a genetic susceptibility in the pathogenesis of growing pains. Furthermore, psychological factors also seem to play a strong role in the onset.

CONCLUSION
The scientific literature about the etiology of growing pains presents heterogeneity and lack of consensus; more studies are needed to understand the genesis of benign musculoskeletal pain syndrome of childhood.

Key words: Growing pains; Benign nocturnal limb pains of childhood; Recurrent limb pains of childhood; Etiology; Pathogenesis

Core tip: Growing pains are benign nocturnal limb pains and the most common cause of musculoskeletal pain in early childhood. Intermittent non-articular pain during the late afternoon or the night with intervals of pain-free days and no objective signs of inflammation are the main clinical features. Despite the etiology of the disease has been widely researched, it is still not fully understood. Lower pain threshold, vascular perfusion changes, anatomical and genetic abnormalities, vitamin D deficiency and psychological factors have been proposed to explain the pathogenesis of growing pain. More studies are needed to understand the complex genesis of the disease.

Citation: Pavone V, Vescio A, Valenti F, Sapienza M, Sessa G, Testa G. Growing pains: What do we know about etiology? A systematic review. *World J Orthop* 2019; 10(4): 192-205
URL: https://www.wjgnet.com/2218-5836/full/v10/i4/192.htm
DOI: https://dx.doi.org/10.5312/wjo.v10.i4.192

INTRODUCTION

French physician Marcel Duchamp first described growing pains as a recurrent leg pain syndrome of early childhood[1]. No evidence has linked growing pains with a phase of especially high growth velocity, which has led physicians to coin new terms for this condition, including "benign nocturnal limb pains of childhood" and "recurrent limb pains of childhood"[2]. However, the term "growing pains" still remains the most commonly used since it has the advantage of not implicating any particular disease and also emphasizes childhood as the period when these pains are most common[3]. The range of prevalence is 2.6% to 49.6%[4,5]. Growing pains are defined by specific clinical features, and Peterson reported diagnostic criteria: intermittent pain that usually occurs once or twice per week late in the afternoon or during the night with intervals of pain-free days; non-articular pain mostly located in the shins, calves, thighs, or popliteal fossa and almost always bilateral; and a lack of pain by the next morning with no objective signs of inflammation[6].

Pain attacks might be very variable in terms of duration, frequency, or severity. Diagnosis is purely clinical since it is mainly based on anamnesis and physical examination with no need for laboratory tests[7]. Although the diagnosis of growing pain seems easy, several entities should be excluded, such as injuries, tumors, and infections, which might mimic the features of growing pains[8]. Growing pains is benign and has a tendency to self-limit once the child grows and reaches adolescence[9]. Thus, treatment might be provided through muscle stretching programs for the quadriceps, hamstrings, and triceps surae groups[9], as well as massaging the affected sites or analgesics[10].

Many studies have tried to investigate the etiology of growing pains over the years without being able to understand it completely. Therefore, the etiopathogenetic mechanisms underlying the phenomena still remain poorly understood. The purpose of this systematic review is to analyze the available literature to provide an update on the latest evidence related to the etiology of growing pains.

MATERIALS AND METHODS

This systematic review was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[11]. The medical electronic databases PubMed and Web of Science were searched by two independent authors (VF and VA) on October 20, 2018. The search string used was "(growing pains OR benign nocturnal limb pains OR musculoskeletal pains) AND (etiology OR pathogenesis) AND (pediatrics)".

The initial titles and abstracts were screened using the following inclusion criteria: studies of any level of evidence, reporting clinical or preclinical results, and dealing with the etiology of growing pains. The exclusion criteria were articles written in other languages or studies with a strict focus on diagnosis, differential diagnosis, or treatment of growing pains. We also excluded all the remaining duplicates and articles dealing with other topics, poor scientific methodology, or no accessible abstract. Study quality was assessed in duplicate by two independent reviewers (VF and VA), and conflicts about data were resolved by consultation with a senior surgeon (PV). Reference lists from the selected papers were also screened. A PRISMA[11] flowchart of the selection and screening method is provided in Figure 1.

RESULTS

A total of n = 440 articles were found. After excluding duplicates, n = 419 articles were selected. At the end of the first screening, we selected n = 51 articles that were eligible for full text reading. After reading the full text, we ultimately selected n = 32 articles that satisfied the criteria. The included articles[12-43] mainly focus on lower pain threshold, reduced bone strength, changes in vascular perfusion, anatomical factors, vitamin D deficiency, genetic susceptibility, psychological abnormalities, and associations. The main findings of the included articles were summarized (Table 1).

Lower pain threshold

Hashkes *et al*[12] hypothesized that growing pains are a non-inflammatory pain syndrome of early childhood that might be associated with a lower pain threshold. The study revealed that the pain threshold was actually lower at all tested points in children suffering from growing pains except for pressure points on the lower back. However, the anterior tibia area is known to be one of the most painful regions among children with growing pains. In a middle-term follow-up study, Uziel *et al*[13] highlighted that patients with persistent growing pains still showed lower pain thresholds at all tested points when compared with both controls and children whose growing pains had resolved.

Changes in vascular perfusion

Oster[14] reported a high prevalence of migraine headaches among children with growing pains, and therefore, Hashkes *et al*[15] tested whether the etiology of growing pains derives from vascular perfusion changes similar to migraine headaches. No significant differences were found among patients with growing pains between painful and painless regions. Furthermore, no significant differences have been identified between children with and without growing pains in both the tibia and femur.

Anatomical factors, reduced bone strength, vitamin D deficiency

Kaspiris *et al*[16] analyzed the association of the onset and intensity of growing pains with some perinatal risk factors. The study revealed that a short gestation period, low Apgar score, low birth length or weight, and lower head circumference were positively correlated with the development of growing pains in childhood. There was also a positive correlation with a higher degree of genu valgum. The use of corticosteroids, high maternal age, and tobacco smoking during pregnancy were found not to be predictive of the disorder. Kaspiris *et al*[17] also reported that children who have been breastfed for fewer than 40 d and those who have not been breastfed at all seemed to have higher chances of developing growing pains.

Many children who undergo observations due to aching legs might also present anatomical abnormalities such as pronated foot posture, which is usually treated using triplane wedges or orthoses. Lee *et al*[18] investigated whether there is an association between foot posture and growing pains and whether shoe inserts were effective in reducing the frequency and severity of growing pain. In this study, 75% of pediatric patients complaining growing pains had overpronated feet and were treated with customized foot orthoses for 3 mo: Pain episodes were significantly reduced, as well as static, dynamic, and functional balancing abilities. Evans[19] revealed that most

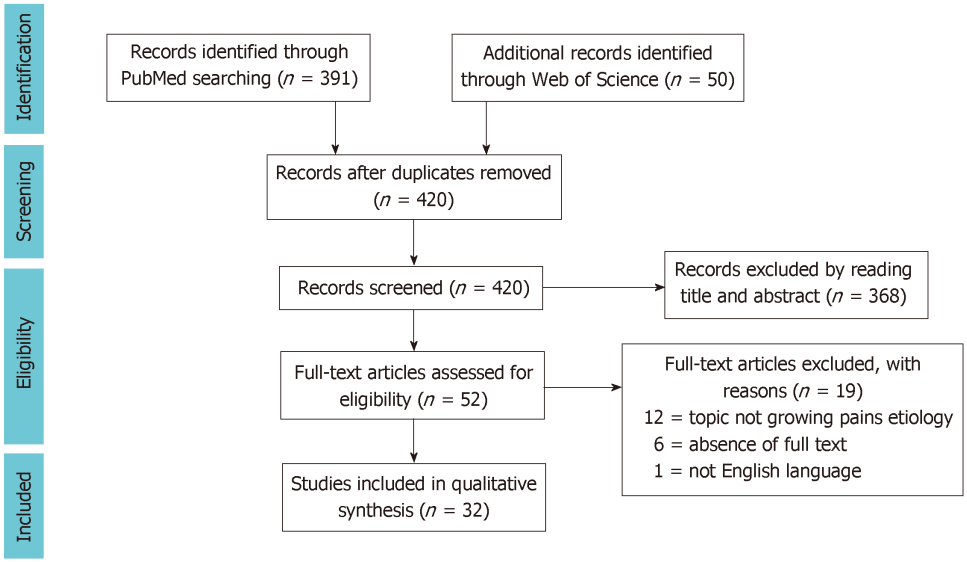

Figure 1 Preferred Reporting Items for Systematic Reviews and Meta-Analysis flowchart of the systematic literature review.

of the patients who used triplane wedges or orthoses had no remaining symptoms after the first period of intervention. After the treatment's withdrawal, symptoms returned in almost all of the patients but decreased in frequency and intensity.

In a subsequent study, Evans and Scutter[20] compared the findings of foot posture and functional health in children aged 4 to 6 years with or without growing pains. However, the results did not reveal any relationship between foot posture, functional health, and growing pains in young children. Viswanathan and Khubchandani[21] assessed the possible association between joint hypermobility and the appearance of growing pains in a cross sectional study. The statistical analysis revealed that joint hypermobility and growing pains were highly related, but to date, this is the only study that has been done to examine this association.

Friedland *et al*[22] hypothesized that growing pains might represent a local overuse or stress syndrome since pain usually occurs late in the day and is often reported on days of activity. The possible association between growing pains and decreased bone strength was investigated by determining the bone speed of sound (SOS) in children with growing pains. Tibial SOS was significantly reduced in both boys and girls suffering from growing pains in comparison to healthy controls.

Uziel *et al*[23] examined children with growing pains who had been previously studied to assess the correlation between bone strength and pain symptoms after 5 years of follow up. Compared to previous study results, bone strength seemed to be significantly increased in both males and females, and there were no differences in bone strength between children whose growing pains continued and those whose pains had resolved. Growing pains still persisted in with a low Z-score (SOS Z-score less than 1 or 2 standard deviations of the population mean).

A cross-sectional study performed by Qamar *et al*[24] showed that patients with vitamin D insufficiency had normal alkaline phosphatase and parathormone levels. Hypocalcemia and hypophosphatemia were found to be more common in patients with vitamin D deficiency, but the findings were not statistically significant. Morandi *et al*[25] showed an increase in 25-OH-D and a decrease in serum levels of parathyroid hormone after 3 mo of oral treatment. Pain intensity was also found to be lower and even disappeared completely in some cases. At 24 mo, vitamin D serum levels were higher than at the beginning of treatment, but pain intensity was found to have decreased further.

Vehapoglu *et al*[26] found that 104 out of 120 patients had vitamin D deficiency, and 25% of them presented vitamin D insufficiency. After 3 mo of oral supplementation with vitamin D, pain intensity decreased significantly. Based on these findings, Park *et al*[27] found lower levels of 25-(OH)D among older patients and also during winter, and the children included in the study mostly suffered from growing pains in winter (41.4%) rather than in summer (12.9%). Insaf[28] estimated serum vitamin D levels in 36 children with growing pains aged 3 to 12 years, and the clinical features improved after oral supplementation with vitamin D. The lowest levels have been reported among children with severe pain, which primarily appeared during the afternoon and was mostly located in the knee and popliteal fossa. Patients who mostly complained

Table 1 The main findings of included case-control and cohort studies

Ref.	Author	Subjects	Etiology	Results
[10]	Pavone *et al* (2011)	30 with growing were enrolled and prospectively followed up for 1 yr. Laboratory tests, including complete blood count, erythrocyte sedimentation rate, and serum calcium and phosphorus levels, were performed in all children	Family history	A family history of growing pains was positive in 20% of patients
[12]	Hashkes *et al* (2004)	In 44 children with growing pains and 46 healthy controls, pain thresholds were measured using a Fisher type dolorimeter with pressure applied to areas associated with increased tenderness in fibromyalgia, control points and anterior tibia	Lower pain threshold	Children with growing pains have more tender points and show lower pain thresholds if compared to healthy children. Growing pains might represent a non inflammatory pain syndrome in young children
[13]	Uziel *et al* (2010)	In the 44 previously studied children with growing pains and in 38 healthy controls, current status of growing pains and other pain syndromes were assessed by parental questionnaires. Pain threshold was also measured by using a Fisher-type dolorimeter	Lower pain threshold	The prognosis of growing pains is benign and with a tendency to self limitation. Growing pains might represent a pain amplification syndrome of early childhood
[14]	Oster *et al* (1972)	635 children were examined annually for five consecutive years or more. Of these, 185 experienced abdominal pain and/or headache for three consecutive years or more while 166 children never had experience of them. Questionnaires were sent to the parents in whom they were ask whether they experienced abdominal pain, headache and/or limb pains in childhood or at the time of investigation	Changes in vascular perfusion	A high prevalence of migraine headaches among children with growing pains have been reported. Recurring abdominal pain, migraine headaches and growing pains are strongly associated and might be part of a reaction pattern based on child's constitution and domestic environment
[15]	Hashkes *et al* (2005)	11 patients with growing pains and 12 healthy controls underwent technetium-99 methylene diphosphate bone scans. The uptake in the blood pool phase, static images, and blood pool phase/static image ratio were measured in the right mid-tibia region (painful among patients with growing pains) and right mid-femur (non-painful). Measurements at painful and painless regions among growing pains children were done. Also children with or without growing pains were compared	Changes in vascular perfusion	There were no significant differences between children with growing pains and healthy controls in the blood pool, static images, and blood pool/static images in all localities. There were also no significant differences among painful regions and non-painful regions in children with growing pains. Growing pains are not associated with vascular perfusion changes in painful regions

[16]	Kaspiris *et al* (2016)	The syudy examined 276 children whose data were collected by using a combination of questionnaires, clinical examinations and medical charts of the children and the obstetric history of the mothers. 78 children presenting growing pains met Peterson's criteria. The tibiofemoral angle and the intermalleolar distance were measured. The perinatal characteristics regarding gestational age, birth weight, length, head circumference, Apgar score, maternal infection, mode of delivery, use of medication and antenatal use of corticosteroids, alcohol or smoking during pregnancy which were based on the medical charts of the children and the maternal obstetrical history were recorded	Genu valgum and perinatal risk factors	Genu valgum severity was a significant factor for growing pains manifestation and increment in frequency and intensity. Perinatal factors including gestational age, Apgar score, head circumference and birth length or weight seemed to be important in growing pains' onset. Conversely, antenatal corticosteroid treatment, increased maternal age and maternal smoking during pregnancy were not predictive of growing pains.
[17]	Kaspiris *et al* (2007)	The study included 532 children, aged 4 to 12 yr. 130 children presented growth pains. Children which had been breastfed were compared to those which were not	Breastfeeding	There is a statistically significant dependence between the presentation of pains and whether the child had been breastfed or not, as well as the duration of breastfeeding during infancy. However, in children with growing pains, breastfeeding does not affect the type or frequency of pain
[18]	Lee *et al*. (2015)	20 patients (seven boys, 13 girls), mean age 9.10 ± 2.32 yr, complaining of musculoskeletal pains in the lower extremities treated with custom made foot orthoses	Foot posture	Twenty children completed the study. Seventeen (75%) had overpronated feet. Significant improvements were noted after 1 and 3 mo in pain degree and frequency, and after 3 mo in balancing ability
[19]	Evans (2003)	8 children complaining of aching legs and with pronated foot posture were treated by wearing triplane wedges or orthoses	Foot posture	In-shoe wedges and foot orthoses are effective in the treatment of young children with growing pains and a pronated foot posture. A relationship between foot posture and growing pains is tenuously inferred
[20]	Evans and Scutter (2007)	180 children underwent foot posture measurements including navicular height, navicular drop, resting calcaneal stance position, foot posture index criteria FPI4 calcaneal inversion/eversion, FPI5 talo-navicular region, FPI6 medial longitudinal arch	Foot posture	No meaningful relationship between foot posture or functional health measures and leg pain in young children does exist
[21]	Viswanathan and Khubchandani (2008)	The study group consisted of 433 children. Joint hypermobility was assessed by using Beighton criteria Children were considered to have growing pains when fulfilling Petersons criteria	Hypermobility	Joint hypermobility and growing pains in schoolchildren are strongly associated. Joint hypermobility might play a role in the pathogenesis of growing pains

[22]	Friedland *et al* (2005)	In 39 children with growing pains, bone speed of sound was measured by quantitative ultrasound in both mid-tibial and radius bones. Patients' findings were compared to norms of healthy controls	Reduced bone strenght	Bone speed of sound was significantly reduced in children with growing pains, especially in painful tibial regions. Growing pains might represent a local overuse (stress) syndrome
[23]	Uziel *et al* (2012)	In 39 previously studied children with growing pains, current growing pains status was assessed by parental questionnaires. Bone strength was measured by using quantitative ultrasound. Controls were normograms based on the measurement of bone speed of sound in 1085 healthy children	Reduced bone strenght	Pain improves parallel to the increase in bone strength. Growing pains might represent a local overuse syndrome
[24]	Qamar *et al* (2008)	100 children with growing pains were investigated for serum total calcium, inorganic phosphorus, alkaline phosphatase, vitamin D3 and parathormone levels. On the basis of serum vitamin D3 level, patients were divided into 3 groups; normal level of vitamin D3, vitamin D insufficiency, vitamin D deficiency	Hypovitaminosis D	Hypovitaminosis D might have a role in pathogenesis of growing pains. Children with unexplained limb pains should be tested for vitamin D status, and treated, if needed
[25]	Morandi *et al* (2005)	In 33 children affected by growing pains pain intensity was evaluated through a questionnaire using the Wong–Baker Faces Pain Rating Scale for pain assessment. Serum 25-OH-D, parathyroid hormone, and alkaline phosphatase levels were also measured. QUS measured both bone density and cortical thickness. After 3 and 24 mo of vitamin D supplementation, pain intensity and laboratory results were re-evaluated. After 24 mo, also QUS parameters we re-assessed	Hypovitaminosis D	After 3 mo of vitamin D supplementation, 25 OH-D levels increased while both parathyroid hormone levels and pain intensity decreased. After 24 mo, parathyroid hormone levels and pain intensity further decreased while QUS parameters improved. A relationship between growing pains, vitamin D levels and bone mineral status might exists
[26]	Vehapoglu *et al* (2015)	In 120 children with growing pains, serum 25(OH)D and bone mineral levels were evaluated at the time of enrollment. The pain intensity of those children with vitamin D deficiency was assessed by the pain VAS. After a single oral dose of vitamin D, the pain intensity was re measured at 3 mo. The 25(OH)D levels and VAS scores before and after oral vitamin D administration were compared	Hypovitaminosis D	Supplementation with oral vitamin D resulted in a significant reduction in pain intensity among those children affected by growing pains who also had hypovitaminosis D
[27]	Park *et al* (2015)	In 140 children with growing pains, levels of serum 25-(OH) D were measured.	Hypovitaminosis D	The high prevalence of vitamin D deficiency or insufficiency in Korean children with nonspecific lower-extremity pains, indicative the association between vitamin D deficiency and growing pains have been found

[28]	Insaf (2015)	36 child with growing pains underwent serum levels of vitamin D measurement at the time of presentation. Patients with low level of vitamin D were incorporated into a prospective cohort study and their pain intensity was measured utilizing a pain VAS. After a single oral or intramuscular dose of vitamin D given to those with low vitamin D levels, pain intensity was re measured at 1 mo. The vitamin D levels and (VAS) scores prior and then vitamin D treatment were compared	Hypovitaminosis D	Many children with growing pain had low vitamin D levels. Treatment with vitamin D resulted in diminishing pain severity in those children with growing pains which also had low vitamin D levels
[29]	Evans and Scutter (2004)	The prevalence of growing pains in children 4 to 6 yr of age in South Australia were reported. A survey of the parents of children, using a validated questionnaire previously developed for this purpose was used. The sample was systematic and randomized across rural and urban regions, with a total of 1445 valid responses achieved	Family history	Family history in growing pains have been reported
[30]	Champion *et al* (2012)	A twin family design study was applied to 88 pairs with at least one twin individual fulfilling criteria for growing pains. Questionnaires for history of growing pains and restless legs syndrome were completed for these twin pairs, their siblings and parents	Family history	Growing pains might have a genetic etiology and a genetic relationship with restless legs syndrome
[31]	Haque *et al* (2016)	Across sectional study included children from four kindergarten schools was carried out. Questionnaires were distributed among the children for indentifying children with limb pain which were selected for further history and clinical examination. Age and sex matched healthy children were selected as controls for comparison of risk factors	Family history	Obesity, over activity and especially family history were identified as growing pains' risk factors
[32]	Naish and Apley (1951)	721 children and their mother were questioned regarding the occurrence of pains. Those children with a history satisfying the criteria were examined at the time and subsequently more fully. The assessment was particularly directed to history of pain, family and personal history and mentality whose assessment included school attainments, school and home behavior. Healthy children were questioned and examined in a similar manner as controls	Psychological disturbances	Few children ad an evident over-reaction to all forms of pain, accompanied by emotional instability. The mother's emotional reactions were also excessive. Emotional disturbances were commoner in children with growing pains. Those children were frequently irritable, nervous, afraid of the dark and also suffering from bad dreams, nightmares, nocturnal enuresis or tics

[33]	Oberklaid *et al* (1997)	160 children with growing pains were compared with a group of 160 healthy controls. In assessing children's behavior and temperament at home and school, mothers and teachers were ask to complete several questionnaires	Psychological disturbances	Children with growing pains were rated by their parents but not by their teachers as having different behavioral and temperamental if compared to healthy controls suggesting the psychological contribution to growing pains' onset
[35]	Makay (2009)	-	Melatonin hormone	The author suggested that some activities including the child's awakening and putting on lights by parents to see what is happening to the child might reduce pain by decreasing melatonin hormone levels
[36]	Lech (2002)	Electrolytes contents of hair taken from 173 children aged 1 to 15 yr and young people aged 16 to 18 yr with growing pains were measured, using the flame atomic absorption spectrometry method, and then compared with those of 108 normal, healthy children	Electrolytes contents	Increased levels of lead and zinc and decreased levels of copper were found in children suffering from growing pains if compared with controls. Magnesium levels for ill children were also enhanced, but in the youngest children, the levels were reduced. Mg/Pb and Mg/Zn ratios were lower and Zn/Cu were higher in the group of children suffering from growing pains than in the healthy children
[37]	Pathirana *et al* (2011)	33 children aged 5 to 12 yr with growing pains were compared to 29 healthy controls. Evidence for peripheral neuropathic disorder was tested by somatosensory testing involved threshold determination and/or response magnitude to non painful stimuli including touch, dynamic brush, cold, vibration, and deep pressure applied to limb and abdominal sites	Somatosensory disorder	Growing pains might be a regional pain syndrome with evidence of mild widespread disorder of somatosensory processing
[38]	Golding *et al* (2012)	Prenatal and postnatal diet, blood measures and variants in fatty acid desaturase genes that influence the metabolism of fatty acids were compared. The study included 1676 children with growing pains at age 8 and 6155 healthy controls	Fatty acids status	No evidence that ω-3 fatty acids status protects against the development of growing pains in childhood have been found
[39]	Ekbom (1975)	A family in which the mother has typical restless legs syndrome and also growing pains since her childhood was observed. Severe growing pains were also showed by her three sons	Restless legs syndrome	Growing pains and restless legs are different conditions
[40]	Rajaram *et al* (2004)	11 children with growing pains were interviewed with their parent to determine if their symptoms of growing pains also met criteria for restless legs syndrome. Those who met clinical criteria for Restless legs syndrome underwent polysomnography whose results were compared to those of 10 healthy controls	Restless legs syndrome	Some children with growing pains also meet diagnostic criteria for restless legs syndrome. A family history of restless legs syndrome have been found among those children

[43]	Evans *et al* (2018)	Foot arches, foot strength, joint mobility, vitamin D and iron levels were examined in 64 children with leg pain and in 13 healthy controls. Children with leg pain were divided into three groups: growing pains, restless legs syndrome, both syndromes are defined for the first time	Increased strength of ankle dorsiflexors and joint mobility were found to be predictive for all types of leg pain. Hypovitaminosis D was detected in 87% of the sample, and anaemia in 13%. Increased body weight, waist girth, and BMI were also found to be associated with leg pain

QUS: Quantitative ultrasound assessment; VAS: Visual analog scale.

of moderate or severe pain before vitamin D supplementation showed a significant reduction of pain severity after treatment, with 31 out of 36 of children being pain free.

Genetic susceptibility

A family history of growing pains has been reported by many authors, such as Evans and Scutter[29] and Pavone *et al*[10] Therefore, Champion *et al*[30] performed a twin family design study to investigate genetic susceptibility to growing pains. There were higher concordance estimates for growing pains in monozygotic twins, as well as a high prevalence of growing pains in at least one parent of the affected children. Haque *et al*[31] assessed the frequency of growing pains in a cross sectional study, which revealed that risk factors were significantly more common in children with growing pains, especially when considering their family history.

Psychological abnormalities

Naish and Apley[32] support the theory that growing pains might have a psychological component. Children suffering from growing pains showed irritability, nervousness, fear of the dark, nightmares, nocturnal enuresis, tics, and an over-reaction to pain. There was also an association with the emotional instability of the mother. Oberklaid *et al*[33] found that children with growing pains tend to have behavior problems and to be more anxious, aggressive, and hyperactive. Lowe *et al*[34] also tried to determine whether the association between growing pains and psychological abnormalities might be related to a familiar pattern. Quality of life and depression levels were found to be the same among parents whose children had growing pains and parents whose children did not. However, mothers of children complaining of growing pains showed higher anxiety levels.

Associations

Makay[35] first proposed an association between the levels of the hormone melatonin and the appearance of growing pains. The author suggested that awakening the child due to pain and turning on the lights to see what is happening to the child are some of the activities that might reduce pain by suppressing melatonin levels. Lech *et al*[36] revealed that electrolyte levels were different between ages and genders. Statistically higher levels of lead and zinc were demonstrated in children with growing pains in comparison to healthy controls. Pathirana *et al*[37] investigated whether growing pains might be associated with some widespread disorders of somatosensory processing. There was no clinical evidence of a peripheral neuropathic pain syndrome and no significant differences in somatosensory test responses between cases and controls at all the tested sites.

Golding *et al*[38] found that the dietary ω-3 intake of mothers and children at 3 and 7 years and the duration of did not significantly differ between children with growing pains and healthy controls. Furthermore, no differences have been found in plasma levels of ω-3 and ω-6 fatty acids at 7 years or in the maternal prenatal red cell levels. Many studies have investigated the possible association between restless leg syndrome and growing pains. Ekbom[39] concluded that restless leg syndrome and growing pains are not the same thing. Rajaram *et al*[40] determined that children with a diagnosis of growing pains might also meet the diagnostic criteria for restless leg syndrome.

Walters *et al*[41] reviewed the scientific literature on the possible association between growing pains and restless leg syndrome with a specific focus on what these two conditions have in common or how they differ. Simakajornboon *et al*[42] also confirmed several studies in investigating the etiological association between restless leg syndrome and growing pains. Many of the studies found similarities and positive overlapping findings between the two syndromes. Recently, Evans *et al*[43] examined

several factors that might be predictors of the onset of leg pains. The study revealed that an increment in joint mobility and the strength of ankle dorsiflexion muscles were predictive of leg pain as well as increased body weight and hypovitaminosis D.

DISCUSSION

The etiology of growing pains remains unknown, and no clear mechanisms have been identified as completely responsible for the manifestation of this pain syndrome. Many theories have been suggested throughout the years, but no one has clarified the major roles in the etiopathogenesis. When the pain threshold was evaluated, it was found to be lower than in healthy controls and persistent[12,13]. These findings suggested that growing pains might be a generalized non-inflammatory pain amplification syndrome of early childhood. Growing pains have also been identified as a local overuse syndrome[22,23]. Children with growing pains had less bone strength than healthy children[22,23]. If local stress were actually able to trigger leg pain, it might explain why painful episodes mainly occur in the late afternoon but not why some patients experience pain in the upper limbs or why pain occurs on days that are not physically demanding[22,23]. One theory proposes that growing pains result from changes in vascular perfusion in painful regions.

Growing pains shares many characteristics with migraine headaches, of which the etiology depends on changes in vascular perfusion. However, no evidence supports this etiological model[14,15]. The anatomical/mechanical theory was first proposed by Hawksley[22,23]. In 2015, the study of Lee *et al*[18] established overloading on supinator foot muscles in patients affected of overpronated foot as a reason of leg pain due to overuse; the use of foot orthoses could be helpful in the treatment of growing pains, according to the previous study of Evans[19], who, at the same times, highlighted only a partially orthoses efficiency. In addition, both studies[18,19] had several limits, as the small size cohort and short follow-up. Moreover, Evans and Shutter[20] found in a cohort of 180 patients only a weak correlation between foot posture and the child's functional health and no longer supports the anatomical theory. In 2016, Kaspiris *et al*[16] found a positive correlation with genu valgum.

Viswanathan and Khubchandani[21] reported a strong association between hypermobility and growing pains given that the simultaneous presence of the two syndromes has been demonstrated in many children.

Vitamin D plays a relevant role in determining bone growth and mineralization[24-28]. Several hypothesis were formulated: In particularly, Morandi *et al*[25] reported, in case of vitamin D deficiency, osteoblasts continue to deposit a collagen rubbery matrix on both endosteal and periosteal surfaces of the skeleton causing an outward pressure on periosteal sensory pain fibers and consequent growing pains; other studies supported that growing pains could be an early manifestation of underlying histological changes in bone caused by osteopenia[24] or due to vitamin D receptors of musculoskeletal and nervous system cells[26,27]. Oral vitamin D supplementation has also been shown to be effective in increasing serum vitamin D levels, reducing the intensity of painful episodes[25,26,28].

A familial pattern in growing pains has been reported by many studies, and it seems to play a role in the etiology of growing pains' as a risk factor[10,29-31]. However, only one study[30] suggested that growing pains are genetically determined. A psychological theory was first proposed by Naish and Apley[32] due to the fact that children with growing pains showed a higher prevalence of behavior and temperament disturbances both at home and school according to parents and teachers. Minor nervous troubles were found to be more common in children presenting growing pains, including irritability, nervousness, nocturnal enuresis, nightmares, and tics.

However, these findings partially disagree with those of Oberklaid *et al*[33], who showed that children with growing pains were rated to be irritable, solitary, unhappy, and distressed by their parents but not by their teachers. This leads to the belief that even if behavioral and temperament abnormalities are present, they are only evident in the eyes of parents and not teachers. However, the importance of the psychological sphere and the family environment seems to be valid because it is also supported by the evidence of high levels of anxiety among the mothers of affected children[34].

Evidence has revealed perinatal risks factors in developing growing pains during childhood[16]. This association suggests that some perinatal risk factors might alter bone metabolism, bone content, and density, and they might be predictors of the manifestation of growing pains in early childhood[16]. Breastfeeding has also been implicated in the prevention of growing pains[17]. The duration of breastfeeding seems to play a role in the appearance of growing pains with durations greater than 40 days

correlating with a lower change of growing pains appearing[17].

The role of melatonin is still controversial and has not been widely investigated. If the association between the nocturnal increase of melatonin and the etiology of growing pains is true, light exposure might represent a therapeutic approach[35]. In the same way, despite some electrolyte alterations having been found among children with growing pains[36], the importance of these results still remains to be investigated further. The pain threshold and the somatosensory response to various non-painful stimuli were tested in children suffering from growth pains[37]. In affected children, the response to some of these stimuli was found to be slightly diffuse rather than specifically localized in the lower limbs compared to healthy controls[37]. This evidence has led to the idea that growing pains might be a regional pain syndrome in which a slight diffusion disorder of the somatosensory processing might be found[37].

Arthritis seems to be influenced by diet and to benefit in particular from the introduction of ω-3 fatty acids, which seems to reduce painful manifestations[38]. However, ω-3 fatty acids do not seem to play a role in either preventing the emergence of the growing pains nor in their treatment[38]. The association between restless leg syndrome and growing pains has long been discussed and verified over the years. Ekbom[39], was the first to investigate this topic but concluded that the two conditions were not associated. Nevertheless, several studies[40-42] do support the theory of a correlation between the two syndromes. There are many common characteristics, such as onset at night and involvement of the lower limbs, but the two syndromes differ in various aspects. Furthermore, the lack of standardized diagnostic criteria for growing pains could make it difficult to distinguish these two entities. For this reason, standardized and universally accepted diagnostic criteria for growing pains need to be established.

Finally, growing pains have recently been associated with not only altered leg-muscle strength, but also with an increase in joint mobility[43]. Children with hypermobile joints report more fatigue with activity than children with normal joint range. Thus, localized biomechanical overload during activity is suggested to lead to the onset of leg pain. In addition, in patients complaining of leg pain, body mass index and body circumference measurements should be performed.

The etiological framing of growing surely represents the most enigmatic aspect of the growing pains phenomenon especially when considering the heterogeneity of the proposed theories and the lack of a univocal consensus. This has led to the belief that different factors, whether individually or in association with each other, might be responsible for the onset of the syndrome. Therefore, it would be highly desirable for other studies to focus on investigating and describing the possible causes and the etiopathogenetic mechanisms underlying growing pains.

ARTICLE HIGHLIGHTS

Research background

Growing pains are the most common cause of musculoskeletal pain in early childhood. The etiopathogenesis of the disease was widely investigated but it is still unknown.

Research motivation

Numerous studies tried to explain the major actors in growing pains etiology but there is a lack of summarize of the evidence.

Research objectives

Analyze the available scientific literature to provide an update on the latest evidence on the etiology

Research methods

Two databases (Pubmed and Science Direct) were systematically searched for relevant article by two independent reviewer. Every step of the review was done according to PRISMA guidelines. Due to article heterogeneity and the topic after data analysis, a descriptive analysis was performed.

Research results

N = 32 articles were included in this systematic review after applying our inclusions and exclusion criteria. Available evidence on growing pains etiology is still inconclusive. Several hypotheses have been researched but none of them was considered decisive.

Research conclusions

After our systematic review we conclude that growing pains etiology rely on different factors, that individually or in association, might be responsible for the onset of the syndrome.

Research perspectives

Further clinical and preclinical studies are strongly encouraged to understand better the possible causes and the etiopathogenetic mechanisms underlying growing pains. Interesting perspective from studies on vitamin D deficit and supplies and anatomic/mechanical theories were found and should be further investigated.

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World Journal of Orthopedics

Submit a Manuscript: https://www.f6publishing.com

World J Orthop 2019 April 18; 10(4): 206-211

DOI: 10.5312/wjo.v10.i4.206

ISSN 2218-5836 (online)

CASE REPORT

Fracture of allograft interbody spacer resulting in post-operative radiculopathy: A case report

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Author contributions: Kyle A, Rowland A, Stirton J and Elgafy H contributed in outlining the manuscript, gathering the data, and writing the manuscript.

Informed consent statement: The patient wrote informed written consent.

Conflict-of-interest statement: None of the authors have any financial or other conflicts of interest that may bias the current study.

Manuscript source: Unsolicited manuscript

Received: January 2, 2019
Peer-review started: January 4, 2019
First decision: January 26, 2019

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Abstract

BACKGROUND

Allograft interbody spacers are utilized during transforaminal lumbar interbody fusion (TLIF) to reestablish anterior column support and disc height. While the TLIF technique offers many improvements over previous surgical methods, instrumentation and bone graft-related complications such as spacer misplacement or migration, screw fracture or misplacement, or rod breakage continue to be reported. The objective of this manuscript is to report on a fractured allograft interbody spacer that displaced into the neural foramen and resulted in impingement on the exiting nerve root that required revision.

CASE SUMMARY

A 50-year-old male had two-level TLIF with immediate post-operative right L5 radiculopathy. Computed tomography scan demonstrated a fractured allograft interbody spacer that displaced into the right neural foramen and impinged on the exiting L5 nerve root. Revision surgery was performed to remove the broken allograft fragments from the right L5 foramen and the intact portion of the spacer was left in place. The right leg L5 radicular pain resolved. At the last follow up 12 mo after the index procedure, computed tomography scan confirmed sound interbody and posterolateral fusion.

CONCLUSION

Displacement of broken allograft interbody spacer following TLIF procedures can result in neurological sequelae that require revision. To avoid such an occurrence, the authors recommend allowing sufficient time for the reconstitution of the graft in saline prior to use to decrease brittleness, to use an impactor size that is as close as possible to the spacer size and meticulous inspection of the cortical allograft spacer for any visible imperfection prior to insertion.

Key words: Transforaminal lumbar interbody fusion; Interbody fusion; Allograft

Revised: February 18, 2019
Accepted: March 16, 2019
Article in press: March 16, 2019
Published online: April 18, 2019

P-Reviewer: Emara KM, Malik H
S-Editor: Wang JL
L-Editor: Filipodia
E-Editor: Wu YXJ

interbody spacer; Postoperative radiculopathy; Graft breakage; Case report

Core tip: Allograft interbody spacers are commonly used in lumbar transforaminal lumbar interbody fusion procedures. A complication previously undocumented in the literature, we report on a fractured allograft interbody spacer that displaced into the neural foramen and resulted in impingement on the exiting nerve root that required revision. At 12 mo post-op, the patient was doing well with computed tomography scan confirming fusion and subsequent removal of the impinging fractured graft fragment. While presumably a rare occurrence, the authors review several technical points to avoid this complication.

Citation: Kyle A, Rowland A, Stirton J, Elgafy H. Fracture of allograft interbody spacer resulting in post-operative radiculopathy: A case report. *World J Orthop* 2019; 10(4): 206-211
URL: https://www.wjgnet.com/2218-5836/full/v10/i4/206.htm
DOI: https://dx.doi.org/10.5312/wjo.v10.i4.206

INTRODUCTION

Transforaminal lumbar interbody fusion (TLIF) is a surgical technique that is utilized for lumbar pathologies for which segmental fusion is indicated[1-5]. This method was developed to improve upon previous surgical methods such as anterior or posterior lumbar interbody fusion. Its unilateral approach can reduce the risk of durotomy, nerve damage, or epidural fibrosis relative to posterior lumbar interbody fusion while decreasing incidence of vascular injury and procedure time relative to anterior lumbar interbody fusion[2,5,7].

Prosthetic allograft interbody spacers are utilized during TLIF procedures to restore anterior column support and disc height in patients with degenerative disc disease and spondylolisthesis[4,8-11]. The demineralized surface of the implant's allogenic bone provides osteoconductivity to aid in osteogenesis and scaffolding for new bone formation along its surface and successful interbody arthrodesis[12,13].

Instrumentation or bone graft-related complications of the TLIF procedure typically involve graft misplacement or migration, screw fracture or misplacement, or rod breakage[14-21]. The authors report on a complication involving a fractured allograft spacer that displaced into the neural foramen impinging on the exiting nerve root during a two-level TLIF. Revision surgery was carried out to remove the broken allograft fragment from the right L5 foramen. To the best of the authors' knowledge, a complication such as this has not been described in the literature.

CASE PRESENTATION

Chief complaints

A 50-year-old male who had previously undergone L5/S1 laminectomy, instrumentation and attempted posterolateral fusion presented to the clinic 2 years after this procedure with chronic low back pain and numbness/pain radiating down his right lower extremity, and to a lesser degree the left lower extremity.

Imaging examination

Computed tomography scan and magnetic resonance imaging confirmed L5/S1 nonunion with hardware loosening and adjacent segment disc degeneration and spinal stenosis. Risks, benefits, and alternative management were discussed and the patient elected to proceed with hardware removal, L4/5 decompression using a right-sided approach, and L4-S1 TLIF and posterior spinal fusion with iliac crest autograft. The procedure was carried out as planned with an allograft interbody spacer impacted into the anterior half of the disk space at both the L4/5 and L5/S1 levels without any obvious intraoperative complication. Soon after awaking from anesthesia, the patient began complaining of right leg radiculopathy along the L5 dermatome. Plain films (Figure 1A and B) showed no obvious complication. Computed tomography scan was then obtained which revealed allograft spacer

fracture with fragment displacement into the right L5 neural foramen (Figure 2A-C).

TREATMENT

The patient was subsequently taken back to the operating room for revision. The broken allograft pieces were identified and extracted (Figure 3). The intact portion of the allograft interbody spacer was left in place. Immediately following the procedure the patient's pain improved, and he was discharged home on post-operative day 2.

OUTCOME AND FOLLOW-UP

Over subsequent clinic visits, right leg L5 radicular pain resolved, and in the last follow-up 12 mo after the index procedure computed tomography scan confirmed sound interbody and posterolateral fusion with no foraminal bone fragment or stenosis (Figure 4A and B, and Figure 5A-C).

DISCUSSION

Complications resulting from the interbody fusion procedures have been well reported since the technique was first described by Harms *et al*[22] in 1982. These complications, however, have typically involved infection, durotomy, bleeding, malpositioned hardware, or pseudoarthrosis[5,11,17,19,21]. Hardware failure reports have been limited to interbody device migration/subsidence, screw fracture, or rod breakage[18,20,23-25]. Saville *et al*[26] showed that when a posterior lumbar interbody fusion spacer is compressed, failure is observed by subsidence of the graft into the upper or lower vertebral bodies and never internally within the graft itself. There have been case studies reporting vertebral fracture following TLIF procedures that may have resulted from the implantation process itself[26].

The interbody fusion technique involves using vertebral endplate shavers to prepare the disc for the fusion. The graft size is determined using trial spacers. Starting with a smaller size trial spacer and under lateral view fluoroscopic guidance, the trial spacer is gradually increased until the most secure fit is achieved, and then selected for the corresponding graft size. The current case reported is unique as part of the graft was sheared off, which resulted in fractured pieces migrating to the right L5-S1 neural foramen, compressing the exiting nerve root, and resulting in right L5 radiculopathy. The remaining bulk of the interbody spacer remained intact and well positioned. No damage to the vertebral bodies was noted.

According to Janssen *et al*[27], allograft spacers have been tested to withstand 25000 N of force compared to the compressive strength of a vertebral body itself as 8000 N. This assumes that the compressive strength of the spacer was equally distributed across the surface of the spacer, as opposed to a localized force applied to a portion of the spacer. As the patient experienced a limited axial load on his vertebrae following surgery, it is unlikely the fracture of the spacer occurred post-operatively. No reports were found of the surgeons experiencing significant difficulty or resistance during the insertion of allograft interbody spacers. Typically, a trial spacer is used to determine appropriate spacer size to achieve good vertebral body distraction and press-fit placement prior to inserting the interbody device. This makes it less likely that a poor interbody spacer size choice can explain the resulting fractured spacer. A system-specific insertion tool was used to implant the spacer decreasing the chance of damage occurring during graft insertion.

There are reports of bone allograft fracture in orthopedic surgery, such as after bone tumor resection. Aponte-Tinao *et al*[28] reported fractured allografts in 19 out of 135 (14%) patients with segmental intercalary bone allograft reconstruction of the lower extremity. The kidney shaped allograft spacer used in the current report is freeze-dried. It is recommended by the manufacturer to reconstitute the graft in saline prior to use to decrease brittleness. After preparation of the disc space, the allograft spacer is inserted into the space with an implant holder, and then an impactor is used to fully seat the spacer into the intervertebral disc space. Possible causes for graft breakage could be one or a combination of the following: insufficient reconstitution of the graft prior to insertion, the impactor used to seat the graft is smaller than the spacer that may lead to shearing of part of the spacer, or the spacer was microscopically cracked prior to its insertion resulting in the graft weakening and breaking.

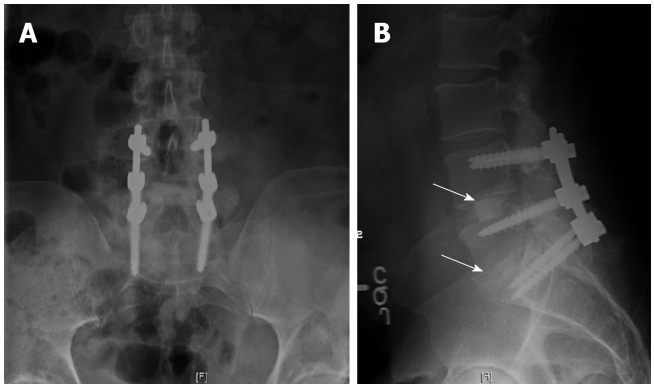

Figure 1 Post-operative radiographs showed good spinal alignment with hardware in good position and interbody spacer at each level is under the midsection of the disc space (arrows). A: Anteroposterior; B: Lateral.

CONCLUSION

Displacement of broken allograft interbody spacer following TLIF procedures can result in neurological sequelae that require revision. Though this complication was found during a TLIF procedure, any lumbar interbody fusion technique is subject to this complication in the event a prosthetic allograft spacer is used. To avoid such an occurrence, the authors recommend allowing sufficient time for the reconstitution of the graft in saline prior to use to decrease brittleness, to use an impactor size that is as close as possible to the spacer size and meticulous inspection of the cortical allograft spacer for any visible imperfection prior to insertion.

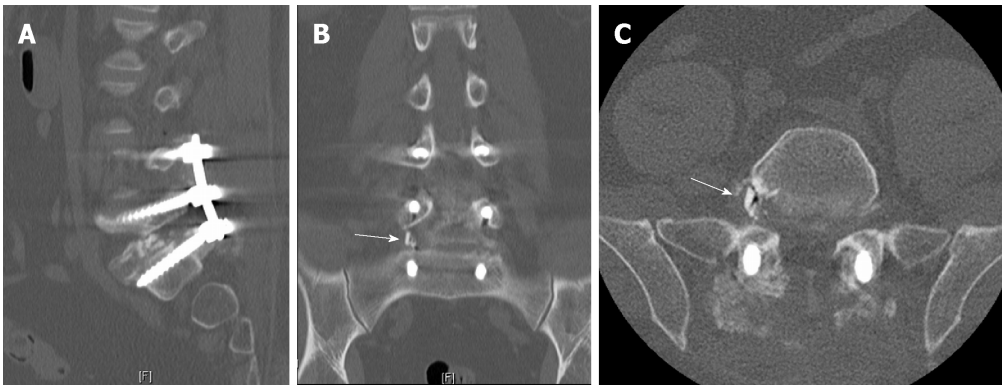

Figure 2 Postoperative computed tomography scan. A: Sagittal view showed the main part of the allograft spacer in the disc space without clear view of broken fragment; B: Coronal view shows obvious fragment (arrow) in the right L5-S1 foramina; C: Axial view also shows obvious fragment (arrow) in the right L5-S1 foramina.

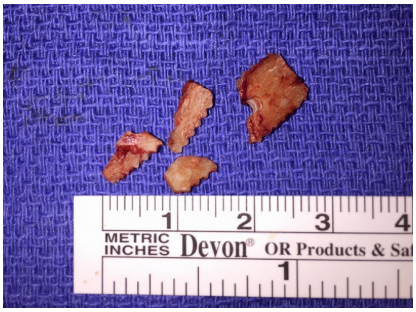

Figure 3 Fragments of prosthetic allograft interbody spacer extracted from right L5 neural foramen.

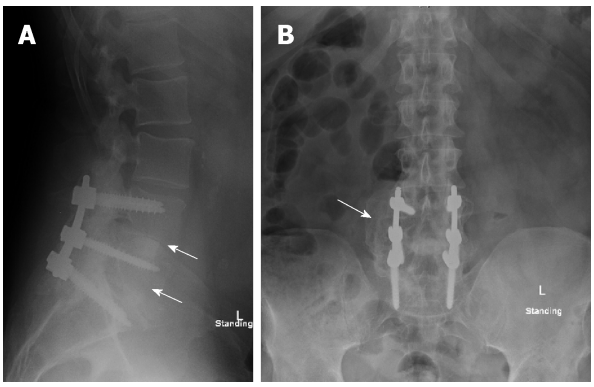

Figure 4 One-year post-operative radiographs showed interbody fusion and posterolateral fusion mass (arrows). A: Anteroposterior; B: Lateral.

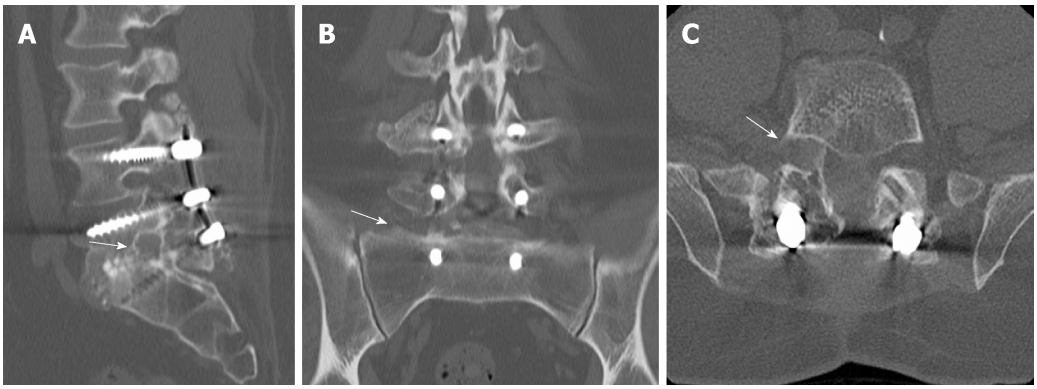

Figure 5 One-year post-operative computed tomography-scan. A: Sagittal, B: Coronal; C: Axial. No foraminal bone fragment or stenosis (arrows).

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World Journal of Orthopedics

Submit a Manuscript: https://www.f6publishing.com

World J Orthop 2019 April 18; 10(4): 212-218

DOI: 10.5312/wjo.v10.i4.212

ISSN 2218-5836 (online)

CASE REPORT

Recalcitrant distal humeral non-union following previous Leiomyosarcoma excision treated with retainment of a radiated non-angiogenic segment augmented with 20 cm free fibula composite graft: A case report

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Author contributions: Gathen M reviewed the literature and drafted the manuscript. Giannoudis PV and Kay S were the patient's surgeons, reviewed the literature and contributed to manuscript drafting; they were also responsible for the revision of the manuscript for important intellectual content; Norris G reviewed the literature and contributed to manuscript drafting; all authors issued final approval for the version to be submitted.

Informed consent statement: Informed written consent was obtained from the patient for publication of this report.

Conflict-of-interest statement: All Authors declare no conflict of interest.

CARE Checklist (2016) statement: The authors have read the CARE Checklist and the manuscript was prepared and revised according the CARE Checklist (2016).

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Abstract

BACKGROUND

Leiomyosacromas in the Extremities are rare malignant smooth muscle tumors. Adjuvant radiation therapy, in combination with wide surgical excision allows the best chance of treatment. During the follow up pathological fractures are common complications that can be accompanied by Implant failure and defect situations that are most challenging in their management.

CASE SUMMARY

We present a case of a 52-year-old female suffering from a pathological fracture of the humeral shaft 10 yr after resection of a Leiomyosarcoma and postoperative radiotherapy. She developed implant failure after retrograde nailing and another failure after revision to double plate fixation. In a two-stage revision, the implants were removed and the huge segmental defect created after debridement was bridged by a compound osteosynthesis with nancy nails and bone cement for formation of the induced membrane. Due to the previous radiotherapy treatment, 20 cm of the humeral shaft were declared devascularized but were left *in situ* as a scaffold. In the second stage, a vascularized fibula graft was used in combination with a double plate fixation and autologous spongiosa grafts for final reconstruction.

CONCLUSION

Manuscript source: Unsolicited manuscript

Received: December 14, 2018
Peer-review started: December 14, 2018
First decision: January 11, 2019
Revised: February 27, 2019
Accepted: March 12, 2019
Article in press: March 12, 2019
Published online: April 18, 2019

P-Reviewer: Gad EH, Salvadori M, Yu B
S-Editor: Ji FF
L-Editor: A
E-Editor: Wu YXJ

This combinatory treatment approach led to a successful clinical outcome and can be considered in similar challenging cases.

Key words: Humerus; Fibular graft; Bone tumor; Osseous defect; Implant failure; Leiomyosarcoma; Case report

Core tip: We present an innovative treatment alternative for segmental bone defects after pathological fracture, tumor resection and radiation of the humerus. A combination of Induced membrane, vascularized fibula graft and double plate fixation was used to bridge a segmental bone defect. Devascularized bone stock was left *in situ* as a scaffold and not resected as usual. The treatment approach led to a successful clinical outcome and can be considered in similar complex cases.

Citation: Gathen M, Norris G, Kay S, Giannoudis PV. Recalcitrant distal humeral non-union following previous Leiomyosarcoma excision treated with retainment of a radiated non-angiogenic segment augmented with 20 cm free fibula composite graft: A case report. *World J Orthop* 2019; 10(4): 212-218
URL: https://www.wjgnet.com/2218-5836/full/v10/i4/212.htm
DOI: https://dx.doi.org/10.5312/wjo.v10.i4.212

INTRODUCTION

After resection of musculoskeletal tumors and following radiotherapy, bone necrosis, osteopenia or radiation-induced neoplasm can be seen[1]. Osteoradionecrosis after radiotherapy often shows a long latent period and the incidence rate is described between 1%-11%. Noteworthy, pathologic fractures become a feared complication and can occur even years after therapy[2].

When pathological fractures occur non-operative therapy rarely provides satisfactory return of function or pain relief. Stabilisation with different methods such as plate fixation, intramedullary nails, external fixators and segmental prosthetic implants can be used with reported osteosynthesis failure rates between 12.2%-22%[3,4]. In cases of segmental bone defects especially those associated with soft-tissue defects, reconstructive options remain limited. Additionally, the presence of previous radiated bone with no inherent angiogenic properties makes this problem even more challenging[3].

In the herein case study, we report the management of a recalcitrant distal humeral non union with implant failures after tumor resection and radiotherapy. Our strategy of optimum fixation accompanied by biological augmentation led to a successful outcome.

CASE PRESENTATION

Chief complaints

A 52-year-old female patient was referred to our institution with persistent pain of the right humerus due to recurrent implant failure and non-union of the right humerus

History of present illness

Ten years previously, the fracture area she was being treated for, had been diagnosed with a grade 2 Leiomyosarcoma. After surgical resection and free tissue transfer (groin flap for covering), a post-operative radiotherapy with 60Gy in 30 fractions was prescribed. The patient then sustained a closed fracture of the right humeral shaft whilst lifting a light object. The fracture was then stabilised with a retrograde nail but fixation failed after 18 mo (Figure 1).

The fracture was next revised to a double plate fixation and biological enhancement was achieved with the implantation of autologous bone grafting harvested from the pelvic iliac crest (Figure 2). However, 14 mo after the revision, the patient presented with non-union associated with implant failure and was referred to our institution (Figure 2).

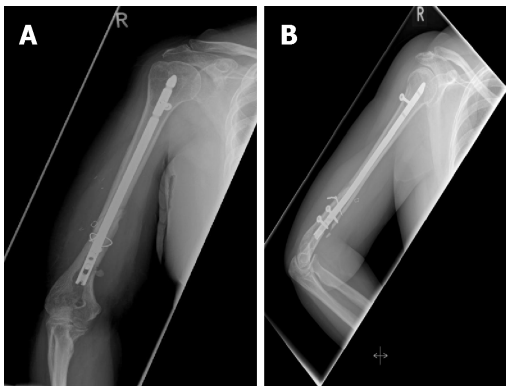

Figure 1 Implant failure after intramedullary nailing. A: AP view of the right humerus after retrograde intramedullary nailing and implant failure; B: Lateral view with displaced distal fragment.

History of past illness
The patient had a free previous medical history.

Physical examination
The patient showed movement dependent pain of the right upper arm. Due to the implanted double plate fixation there was no instability. After radiation and previous surgery the skin was compromised by massive scar tissue formations. The patient showed no clinical signs of infection and no neurological deficits.

Laboratory examinations
Blood analysis as well as urine analysis were normal. Electrocardiogram, chest X-ray and arterial blood gas were also normal.

Imaging examinations
A staging computed tomography and magnetic resonance imaging scan of the upper arm showed no local recurrence or metastatic disease.

FINAL DIAGNOSIS
Distal humeral non-union associated with implant failure after resection of a Leiomyosacroma and following radiotherapy.

TREATMENT
Due to the previous radiotherapy and concerns about the possibility of low-grade infection, a two-stage revision was planned. Initially, the implants were removed and the non union area was debrided with multiple tissue samples sent to microbiology. A defect of 2.5 cm was created. Temporary stabilisation was performed using a compound osteosynthesis with nancy nails and bone cement followed by wound closure (Figure 3). The notes from the referral hospital center were requested in order to identify the extent of the previous bone radiated area. Two out of the 5 culture specimens grew staphylococcus aureus and the patient was treated with appropriate antibiotics for a period of 6 wk.

During the second stage and despite the induction of the induced membrane (IM) (which can promote bone regeneration), it was felt that autologous bone grafting in isolation would not be successful since the bone edges around the non union site were lacking angiogenic capacity and healing potential[5]. The zone of the previous radiotherapy was assessed to have been from the olecranon fossa to 20 cm proximally just below the lesser tuberosity. In view of the extent area of radiated bone it was decided to leave this section of humerus *in situ* and considered it in our reconstruction strategy as a scaffold.

A composite fibula vascularised graft was harvested from the ipsilateral tibia 20 cm in length and the vascular graft was connected to the brachial artery. The fibula graft was placed anterior laterally and two plates were used for stabilisation. One to provide continuity to the distal and proximal radiated humeral segments and the other to stabilise the fibula on the humerus (Figure 4). The previous created bone defect was addressed by shortening of the humeral shaft by 2.5 cm. Autologous bone

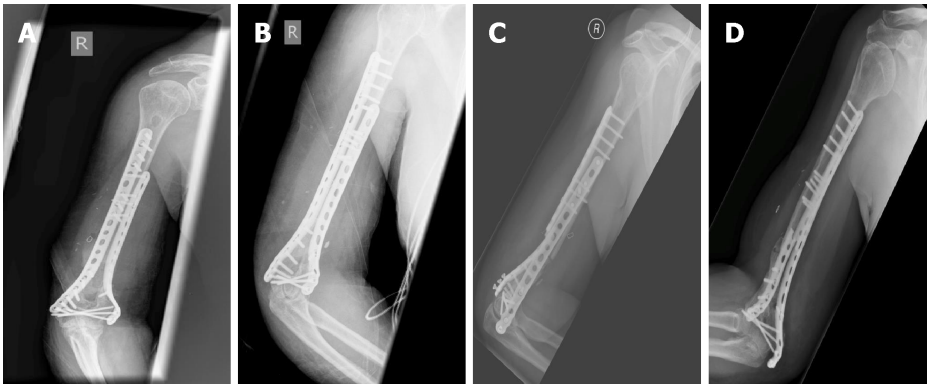

Figure 2 Second implant failure after ORIF. A: AP view of the right humerus shortly after revision using double plate fixation; B: Lateral view; C: AP view 14 mo after revision to ORIF; D: Lateral view showing implant failure.

graft was implanted distally.

OUTCOME AND FOLLOW-UP

After a period of 6 months osseous healing was observed. Twelve months following reconstruction one plate was removed due to soft tissue irritation. At final follow up (2 years later), hypertrophy of the fibula graft was noted with restoration of right arm function (Figure 5). The range of motion was full flexion, minus 20^0 of full extension and full supination pronation of the forearm. Shoulder movements were full and pain free.

DISCUSSION

Pathological fractures of the humerus are common and associated with high re-operation rates[6]. Sarahrudi *et al*[4] analysed the treatment of 39 patients with pathological humerus fractures and reported a complication rate of 14.6%. They found intramedullary stabilization to be most reliable for fractures of the diaphysis and ORIF preferable for the treatment of metaphyseal fractures and for patients with a solitary metastasis[4]. In a different study, a cemented hemiprosthesis for proximal fractures was found most suitable and intramedullary nailing for lesions in the diaphysis[6].

Previous radiotherapy in the medical history of pathological fractures and particularly non union complicates treatment options and fracture healing potential due to radiation-induced osteopenia and loss of bone vitality. In our case there were 2 previous attempts of reconstruction without success. Although the masquelet technique was employed[5], it was felt that even with the presence of the IM *in situ*, which is highly vascular, containing growth factors and osteoprogenitor cells, the chance of healing was low. Consequently, it was deemed essential to bring vascularity to the region and structural support with the free fibula composite vascularised graft being the ideal option to bridge the avascular area of the humerus. Moreover, the retainment of the humeral avascular segment would simplify the reconstruction process by acting as a scaffold within the local environment. The double plate approach provided adequate mechanical stability until evolution of healing occurred.

Segmental defects in long bones are challenging tasks especially when further factors such as poor soft tissue, osteonecrosis or infections are present. A variety of techniques and their combinations have been described such as bone transportation, IM Technique, autograft bone grafting, and megaprosthesis[7-9].

Using a vascularized fibula graft is a highly sophisticated technical procedure with potential complications including non-union, graft fracture and donor site morbidity. Advantages include the straight configuration and dual vascularity (endosteal and periosteal). The method provides shorter duration than therapy with bone transportation and a tendency for hypertrophy in response to microscopic stress fractures[10,11]. The IM Technique is described for diaphyseal defects from 5 cm to 25 cm allowing formation of bone by means of endochondral ossification with high union rates of 90%[12].

In our case we employed a combination of techniques, including the IM, au-

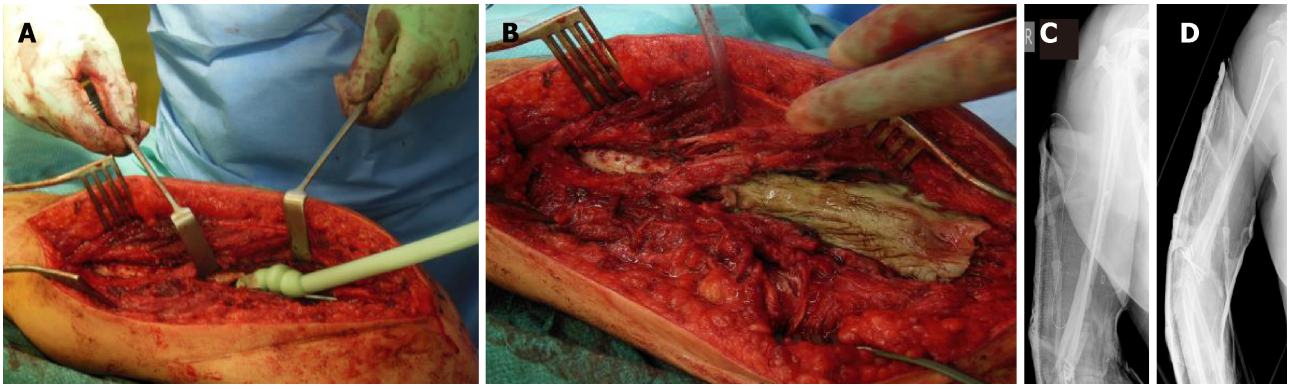

Figure 3 Induced membrane technique. A: Intraoperative picture of the cement application to the humeral defect site after implantation of two nancy nails; B: Hardened cement used as a temporary space holder; C, D: AP and lateral X-ray after first surgery and implantation of two nancy nails.

tologous bone grafting for the distal humerus segment, a composite vascular fibula graft and retainment of the radiated-devitalised humeral segment as a scaffold. One may argue that it is controversial that the avascular bone was left *in situ* because it is a widely accepted concept to resect avascular bone tissue completely[13,14]. However, this approach was found useful, eliminating the need of considering the use of a mega-prosthesis and facilitating the reconstruction process by acting as a bridge allowing implantation of the fibula graft.

CONCLUSION

We believe that the presented management of treatment of a recalcitrant humeral non-union with a background of previous pathological fracture following radiotherapy for leiomyosacroma is a promising alternative and should be considered as an option of treatment, when contemplating reconstruction of such complex cases.

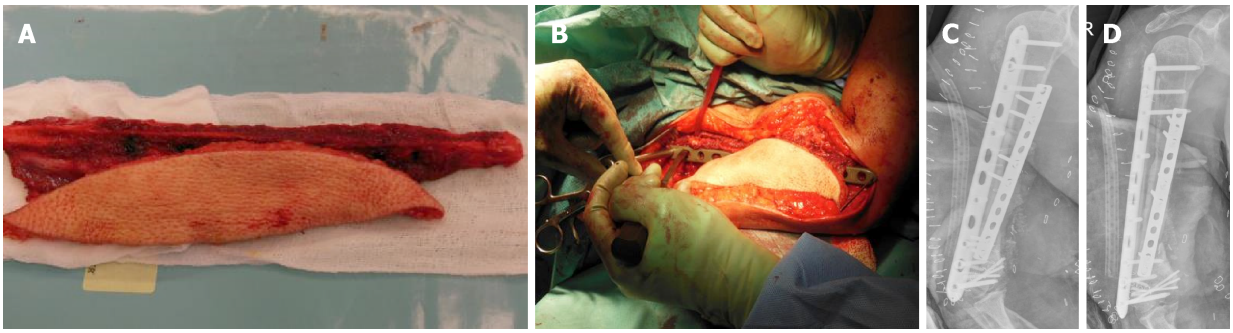

Figure 4 Vascularized fibula graft and plate fixation. A: Vascularized fibula graft (VFG) after harvesting. B: Final plate fixation for stabilization of the VFG and bridging the defect. C, D: Lateral and AP X-ray after double plate fixation and combined vascularized fibular graft.

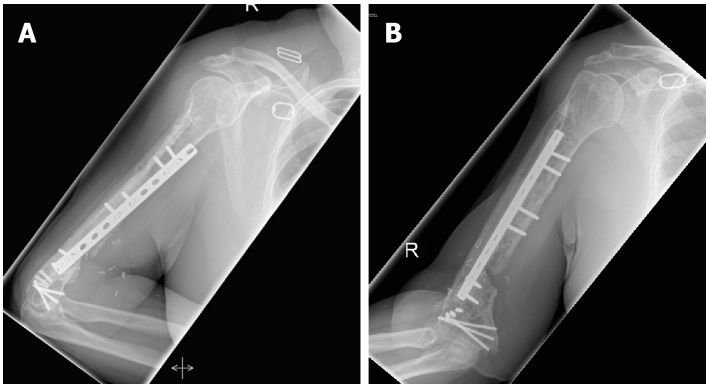

Figure 5 Final result. A: Final lateral X-ray after removal of one plate and osseous integration of the VFG; B: AP view with a good sight on the integrated VFG. VFG: Vascularized fibula graft.

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