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Can autologous bone culture predict spinal fusion capacity?

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Abstract The capacity of the individual patient to initiate osteoblast proliferation as a predictor for successful lumbar spinal fusion has not yet been reported. The objectives of this study were, first, to analyze the relationship between in vitro osteoblast proliferation and clinical bony fusion in the individual patient in order to predict the fusion outcome and, second, to measure the effect of preoperative tobacco smoking on osteoblast proliferation. Sixty-one patients (mean age 46 years) underwent posterolateral lumbar fusion in the period 1994–1995. Thirty-eight patients received CD pedicle screw implants and 23 received posterolateral fusions alone. During surgery, autogenous iliac bone was harvested and 1 g of trabecular bone without blood or bone marrow was then isolated for cell culturing. The cultures were classified as excellent (confluence within 4 weeks), good (confluence between 4 and 6 weeks) and poor (no or poor growth). Spine fusion was evaluated by two independent observers from plain anterior-posterior, lateral, and flexion/extension radiographs taken 1 year post-operatively, and the functional outcome was measured by the Dallas Pain Questionnaire (DPQ). Twenty-three patients had excellent, 19 good,

and 19 poor in vitro osteoblast proliferation. Bony fusion was obtained in 77% of patients: 83% in the CD instrumentation group and 70% in the non-instrumentation group (NS). There was no significant correlation between osteoblast proliferation and spinal fusion or functional outcomes when analyzing the CD instrumentation and non-instrumentation groups together or separately. Elderly patients had a significantly poorer osteoblast proliferation than younger patients ($P < 0.008$). Preoperative tobacco consumption had no discernible effect on osteoblast proliferation, and no correlation between smoking and fusion was found. Further refinement of autologous osteoblast culturing may provide a biological tool for selection of patients who require biological enhancement of their bone fusion capacity. The poorer osteoblast proliferation related to advanced age supports the important negative biological influence of age on bony fusion. However, with more sensitive testing and better discrimination, other results are possible – or can in any event not be excluded.

Key words Spinal fusion · Autogenous bone graft · Osteoblasts · Prediction · Culture

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Introduction

Pseudarthrosis must be anticipated in 5–25% of patients undergoing spinal fusion [35], but the etiology of spinal pseudarthrosis is controversial. Important local factors (mechanical environment, fusion site preparation, blood supply, bone graft source and quantity) are believed to operate in concert with systemic factors [1].

Supplementary rigid pedicle screw fixation in posterolateral lumbar fusion has been claimed to offer several theoretical advantages when compared with fixation by non-instrumented techniques. These include better correction of deformity and higher fusion rates by improving the mechanical milieu [12]. However, three recently published randomized prospective studies have demonstrated a significant improvement in functional outcomes of posterolateral lumbar fusion, which were not achieved by use of supplementary pedicle screws alone [11, 22, 33]. A better understanding of patient-related factors may improve the clinical outcome through better patient selection. So far, a number of individual factors have been found to be accompanied by inferior spinal fusion, these include osteoporosis, smoking, hormones (thyroid, corticosteroids, estrogens), and various drugs (methotrexate, adriamycin, nonsteroidal anti-inflammatory drugs) [1].

It has been possible to cultivate bone cells *in vitro* for more than a decade [27]. The purpose has mainly been to analyze the effect of hormones and growth factors on bone cell metabolisms [13, 15, 16, 21]. Studies have shown a marked reduction in the number and *in vitro* activity of stromal osteogenic cells from old animals [9, 13, 28, 34]. It appears that the gradual bone loss associated with old age results from a decreased osteoblast recruitment for bone-formation activity rather than from increased osteoclastic activity [24, 30]. On the other hand, the accelerated bone loss associated with decreased estrogen level, as in postmenopausal osteoporosis, appears to be caused by increased osteoclastic activity [24, 30]. The aim of this study was firstly to analyze the relationship between *in vitro* osteoblast proliferation and clinical bony fusion in the individual patient in order to predict the fusion outcome and, secondly, to measure the effect of preoperative tobacco smoking on osteoblast proliferation.

Materials and methods

From November 1993 to February 1995, 63 consecutive recipients of lumbar posterolateral spinal fusion had 3 g of autogenous iliac bone graft harvested for *in vitro* culturing in our bone cell laboratory. Two cultures became infected within a few days and were excluded.

This study therefore comprised 61 patients, 22 men and 39 women, with a mean age at surgery of 46 years (range 21–73 years). The preoperative diagnosis was disc degeneration (34 patients) or isthmic spondylolisthesis (27 patients). Twenty-three patients had undergone previous spinal surgery. Thirty-eight patients received CD pedicle screw implants (25 on one level, 9 on

two levels and 4 on three levels) and 23 received posterolateral fusions alone (15 on one level, 5 on two levels and 3 on three levels) (Table 1). Supplementary pedicle screw fixation was generally used in revision surgery and where major decompression was needed.

All the data were prospectively collected by the surgeons responsible, who completed registration sheets before and after surgery, at discharge, and at 1 year follow-up in the outpatient clinic. All the data were continuously stored in our spine surgery database. Fusion was evaluated by two independent observers from plain anterior-posterior, lateral, and flexion/extension radiographs taken 1 year postoperatively. Each level and each side were judged separately and continuous trabecular intertransverse bony structure on at least one side indicated union at that level. If fusion was done on two levels, union on both levels was required for classification as complete fusion. Complete union was only obtained if the two observers were in agreement. Incomplete union indicates suboptimal quality at one or more levels, including fusion mass hidden behind the instrumentation, and non-union indicates a definitive lack of fusion at one or more of the intended levels.

Functional outcome was assessed by means of the Dallas Pain Questionnaire (DPQ) [3, 14]. The DPQ assesses the functional impact of chronic spinal pain in four categories: daily activities, work-leisure activities, anxiety-depression, and social concerns. The questionnaire was completed by the patients, independent of the surgeons, the day before the operation and at 1 year after surgery and was scored by an independent observer (secretarial assistance). Smoking habits preoperatively and during the first 6 months postoperatively were assessed by a questionnaire.

Cell culturing

The iliac crest trabecular bone graft was always harvested from the proximal iliac crest as uniformly as possible. The bonegraft used for cell culturing was randomly taken from the bone graft harvested for the spinal fusion. It was then placed in Earles Minimal Essential Medium (Life Technologies). The trabecular bone was cut into 1- to 2-mm chips, washed by PBS (phosphate-buffered saline) and then treated with type IV collagenase (Sigma) for 120 min as described previously [16, 27]. After washing again with PBS, 1 g of bone chips was placed in a 80 cm² culture flask (Nunc, Denmark) with Earles Minimal Essential Medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 50 µg/ml streptomycin and 100 µg/ml penicillin G. The medium was changed weekly and the cells were grown to confluence. Collagenase treatment and culturing were always identical. Stability of the osteoblastic phenotype in the cell cultures was evaluated in every ten cultures. When cells had reached confluence, they were characterized for the osteoblastic phenotype by type I collagen and osteocalcin immunostaining. Responsiveness to vitamin D₃ stimulation in terms of increased alkaline phosphatase activity and osteocalcin production and responsiveness to PTH stimulation in terms of increased intracellular cAMP levels were also analyzed. All the graft bone for culturing was prepared in exactly the same way.

The proliferation capacity of the cultured osteoblasts was evaluated by the cells' ability to reach confluence in culture. This was semiquantified by classifying a culture as excellent if confluence was reached within 4 weeks, good if confluence was reached between 4 and 6 weeks, and poor if confluence was reached after more than 6 weeks or not reached at all (Fig. 1).

Our department has been routinely working with osteoblast cultures since 1993. A trained laboratory technician analyzes the cell cultures and scores them after the department's standard procedures into the above three categories [16].

The study was approved by the National Medical Ethical Committee.

Table 1 Patient data (*Deg* disc degeneration, *Listhesis* isthmic spondylolisthesis, *Previous* previous lumbar spinal surgery)

No.	Sex	Age	Diagnosis	Op. level	Previous	+/- Implants	Decompression	Fusion ^a	Complications
1	F	69	Deg.	L2-L5	—	+	+	1	No
2	M	51	Listhesis	L5-S1	—	+	+	1	No
3	M	53	Listhesis	L3-S1	—	+	—	1	No
4	M	48	Deg.	L4-L5	—	—	+	1	No
5	F	38	Listhesis	L5-S1	—	—	—	1	No
6	F	29	Listhesis	L5-S1	—	+	+	1	No
7	F	56	Deg.	L2-L5	+	+	+	1	No
8	F	45	Deg.	L4-S1	—	—	—	2	No
9	M	56	Listhesis	L5-S1	—	+	+	1	No
10	M	58	Deg.	L4-L5	—	+	—	1	No
11	F	49	Listhesis	L4-L5	—	+	+	1	No
12	F	41	Deg.	L5-S1	+	—	+	1	No
13	F	60	Deg.	L5-S1	—	+	+	1	No
14	M	47	Deg.	L5-S1	—	+	—	1	No
15	F	73	Deg.	L2-L5	—	—	+	2	No
16	F	48	Deg.	L5-S1	+	—	—	1	No
17	F	44	Deg.	L3-S1	+	+	—	·	No
18	F	52	Listhesis	L5-S1	—	+	+	1	No
19	F	49	Listhesis	L5-S1	—	+	+	1	No
20	F	62	Deg.	L2-L5	—	—	+	1	No
21	M	50	Deg.	L4-S1	+	+	+	2	No
22	F	48	Deg.	L4-S1	+	+	+	1	No
23	F	44	Deg.	L5-S1	+	+	+	1	No
24	F	58	Deg.	L5-S1	—	—	—	1	No
25	F	30	Listhesis	L5-S1	+	+	+	1	No
26	M	55	Deg.	L4-L5	—	+	+	1	No
27	F	48	Deg.	L4-S1	—	+	+	3	No
28	M	21	Listhesis	L5-S1	—	—	—	1	No
29	M	27	Listhesis	L5-S1	—	+	+	2	Broken screw ^b
30	F	42	Listhesis	L5-S1	—	—	—	2	No
31	M	50	Deg.	L4-L5	—	—	+	2	No
32	F	45	Deg.	L4-S1	+	+	+	1	No
33	M	39	Deg.	L4-S1	+	—	—	1	No
34	F	44	Listhesis	L5-S1	+	+	+	1	No
35	F	50	Deg.	L4-S1	—	—	—	1	No
36	M	24	Listhesis	L5-S1	—	—	—	1	No
37	F	48	Deg.	L4-S1	+	—	+	1	No
38	M	46	Deg.	L5-S1	+	+	+	·	No
39	F	37	Listhesis	L5-S1	+	+	—	2	No
40	M	44	Listhesis	L5-S1	—	+	—	1	No
41	M	45	Deg.	L5-S1	+	—	—	1	No
42	F	35	Listhesis	L5-S1	+	+	+	1	No
43	F	22	Listhesis	L5-S1	—	+	—	1	No
44	F	41	Deg.	L5-S1	+	—	+	1	No
45	F	36	Listhesis	L5-S1	—	+	+	1	No
46	F	37	Listhesis	L4-L5	—	+	—	2	No
47	F	51	Deg.	L2-L4	—	+	+	1	No
48	M	49	Listhesis	L2-L3	—	+	+	1	No
49	F	48	Listhesis	L5-S1	—	+	—	3	No
50	F	23	Listhesis	L5-S1	—	+	+	1	No
51	M	62	Deg.	L5-S1	+	—	+	3	No
52	F	51	Listhesis	L5-S1	+	+	+	1	Infection ^c
53	M	57	Deg.	L4-L5	+	+	+	1	No
54	M	40	Deg.	L5-S1	+	+	+	1	No
55	F	37	Deg.	L4-L5	+	—	+	1	No
56	F	59	Deg.	L3-S1	—	—	+	·	No
57	M	57	Listhesis	L4-L5	—	+	+	1	No
58	F	51	Deg.	L5-S1	+	—	+	1	No
59	F	52	Deg.	L4-L5	—	—	+	1	No
60	M	40	Listhesis	L5-S1	—	+	+	1	No
61	F	24	Listhesis	L5-S1	—	—	—	2	No

^a 1 = Complete union, 2 = incomplete union, 3 = non-union, · = non complier^b One broken sacral screw^c Superficial infection

Fig. 1 Osteoblast grown to confluence visualised in phase-contrast microscopy

Statistics

The Pearson chi-squared test, Mann-Whitney-Wilcoxon Rank Sum Test, and Kendall's tau test were applied. Kendall's tau test was used for calculating the correlation between cell proliferation and age with a two-tailed 1% limit of statistical significance. A 5% two-tailed limit of statistical significance was used for all other calculations. SPSS/PC was the statistical software used.

Results

All cultures selected for characterization demonstrated positive immunostaining for osteocalcin and collagen type I in more than 90% of the cells. Also vitamin D₃ stimulation and PTH stimulation resulted in increases in alkaline phosphatase activity and intracellular cAMP levels. Twenty-three patients had excellent, 19 good, and 19 poor in vitro osteoblast proliferation. Elderly patients had a significantly poorer osteoblast proliferation than younger patients ($P < 0.008$) (Fig. 2). No significant influences of sex, diagnosis, or preoperative walking distance were found on osteoblast proliferation (Table 2).

The radiological follow-up included 58 patients (95%). Three patients left the study as non-compliers (did not attend the clinic for the follow-up). Overall complete radiological bone union was obtained in 77% of the patients: 83% in the CD instrumentation group and 70% in the non-instrumentation group (NS). There was no significant correlation between osteoblast proliferation and spinal fusion when analyzing the CD instrumentation and non-instrumentation groups together or separately (Table 2). When analyzing 'complete union' compared to 'incomplete and non-union' together there was no significant difference in osteoblast proliferation. However, none of the three patients (one man and two women) with 'non-union' was classified as having excellent osteoblast proliferation.

Osteoblast proliferation

Fig. 2 Osteoblast proliferation in relation to patient age. A poorer osteoblast proliferation was found in relation to increasing age ($P < 0.008$). The 95% confidence intervals were: excellent (36–46 years), good (42–49 years) and poor (45–57 years). The box-plots show range, median, 25th percentile (lower box) and 75th percentile (upper box)

Table 2 In vitro osteoblast proliferation in relation to sex, diagnosis, walking distance, spinal fusion and preoperative tobacco consumption

	Excellent		Good		Poor		P-value
	n	(%)	n	(%)	n	(%)	
<hr/>							
Sex							
Male	9	(41)	6	(27)	7	(32)	NS
Female	14	(36)	13	(33)	12	(31)	
Diagnosis							
Disc degeneration	11	(32)	11	(32)	12	(36)	NS
Spondylolisthesis	12	(44)	8	(30)	7	(26)	
Walking distance							
< 500 m	8	(33)	7	(29)	9	(38)	NS
501 m < x < 1000 m	5	(42)	4	(33)	3	(25)	
> 1001 m	9	(39)	8	(35)	6	(26)	
Spinal fusion							
CD-instrumentation							
Complete union	9	(82)	13	(81)	8	(89)	NS
Incomplete union	2	(18)	2	(13)	0	(0)	
Non-union	0	(0)	1	(6)	1	(11)	
No instrumentation							
Complete union	8	(89)	2	(67)	6	(60)	NS
Incomplete union	1	(11)	1	(33)	3	(30)	
Non-union	0	(0)	0	(0)	1	(10)	
Smoking tobacco							
Yes	8	(29)	11	(39)	9	(32)	NS
No	8	(35)	7	(30)	8	(35)	
Tobacco consumption							
< 15 cigs/day	6	(30)	9	(45)	5	(25)	NS
> 15 cigs/day	2	(29)	1	(14)	4	(57)	

Table 3 Dallas Pain Questionnaire (DPQ) scores, expressed as median (range), after posterolateral spinal fusion in relation to in vitro osteoblast proliferation from autologous bone graft. No significant differences were found between the groups

	Daily activity	Work-leisure activity	Anxiety-depression	Social interest
Overall				
Excellent (<i>n</i> = 20)	27 (-21; 84)	23 (-40; 80)	15 (-15; 55)	5 (-15; 40)
Good (<i>n</i> = 17)	24 (-30; 60)	15 (-5; 50)	10 (-40; 60)	5 (-35; 60)
Poor (<i>n</i> = 15)	20 (-21; 57)	-8 (-40; 45)	5 (-30; 55)	0 (-45; 35)
CD instrumentation				
Excellent (<i>n</i> = 11)	27 (-21; 84)	25 (-40; 80)	20 (-10; 55)	10 (-15; 40)
Good (<i>n</i> = 12)	29 (-30; 60)	13 (-5; 50)	10 (-40; 40)	10 (-25; 60)
Poor (<i>n</i> = 8)	24 (-21; 57)	-5 (-20; 20)	20 (-30; 55)	-5 (-45; 35)
No instrumentation				
Excellent (<i>n</i> = 9)	27 (-18; 45)	20 (-35; 55)	5 (-15; 20)	5 (-10; 30)
Good (<i>n</i> = 5)	21 (-18; 24)	15 (-5; 15)	30 (-30; 60)	0 (-35; 15)
Poor (<i>n</i> = 7)	0 (-27; 30)	3 (-40; 45)	3 (-5; 40)	0 (-35; 25)

They were 48, 49, and 62 years of age and one had previously undergone spinal surgery. Two of the patients smoked for the first 6 months postoperatively and all 3 patients had preoperative disc degeneration. Fifty-two patients (85%) answered the DPQ at 1-year follow-up. The non-responders were equally distributed between the three cell culture groups (three excellent / two good / four poor). We found no correlation between osteoblast proliferation and functional outcomes in any of the four DPQ categories, either for the instrumented or the non-instrumented group (Table 3).

At 1-year follow-up, 53 patients (87%) answered the questionnaire concerning smoking habits preoperatively and during the first 6 months postoperatively. Sixty-five percent of the patients had been heavy tobacco smokers (more than 15 cigarettes/day) earlier in their life. Forty-seven percent had smoked tobacco in the last 3 months preoperatively, and 77% smoked more than 15 cigarettes a day at the time of surgery. Forty-one per cent smoked during the first 6 months postoperatively even though they were advised not to. Preoperative tobacco consumption had no influence on osteoblast proliferation (Table 2).

There were no clear correlations between pre- and postoperative tobacco consumption and spinal fusion rate.

Discussion

One way to improve the overall outcome of spinal fusion would be identifying patients at risk of an inferior outcome, thereby improving patient selection. Until now, patients at risk of an inferior outcome have mainly been identified by looking at data such as sex, age, diagnosis, walking distance and pain scores, in the form of questionnaires [10, 17–20, 37] and pain drawings [25,36].

In vitro human osteoblast cultures have been performed since the mid 1980s [27], but no previous study has addressed the hypothesis that in vitro osteoblast proliferation could predict spinal fusion.

The present cell culture semi-quantitative classification system as in vitro predictor of in vivo bone healing has not been previously published. The classification is based on our and others' osteoblast in vitro laboratory experience of osteoblast growth in culture [16]. The same trained laboratory technician prepared and analyzed all the cell cultures. This standard procedure has its limitations and can be criticised. However, the fact that age correlated with osteoblastic growth in our system indicates that the classification can be used to evaluate healing ability. Numerous analyses of osteoblastic activity exist and could also be used as predictors of arthrodesis, e.g., cell proliferation measured by thymidine incorporation, matrix protein synthesis such as type 1 collagen and osteocalcin production, alkaline phosphatase activity as a measure of mineralising activity.

In the present study we found no significant influence of osteoblast proliferation on radiological outcome in the CD instrumentation group or non-instrumentation group. It is noteworthy, however, that none of the three patients with non-union were classified as having excellent cell proliferation. In a pig study, 18 mini-pigs underwent bilateral posterolateral fusion and 2 g of autogenous iliac bone graft was harvested and used for in vitro cell culture [4]. After 3 weeks the cells were counted by quantitative assessment techniques (a point grid) and correlated with the spinal fusion rate after 12 weeks, tested mechanically, by X-rays and three-dimensional CT. All four animals with non-union had a significantly lower cell proliferation than the animals with union.

There is an ongoing debate concerning the validity of radiographic evaluation of the fusion mass. We believe that it is important to attempt to evaluate the solidity of the fusion. The classification system used in this study has been evaluated in a not yet published study, by Christensen and colleagues, and has shown acceptable interobserver agreement (83–90%) and intraobserver agreement (87–97%).

There was no correlation between osteoblast proliferation and functional outcome. In analyzing and recording

pain, function, and disability, both before surgery and at follow-up, we have since 1989 routinely made use of the Dallas Pain Questionnaire, which has been shown to be a valid instrument for clinical assessment of low back pain [32].

We found a negative correlation between high age and osteoblast proliferation capacity. One explanation could be that the number of osteogenic stem cells is decreased in elderly patients, and this may be as important for successful bone fusion as mass of transplanted autologous bone. As previously mentioned, this correlation has been shown in several animal studies [9, 13, 28, 34]. To our knowledge no study has been published showing a clear correlation between patient-related factors such as age and the ability of autologous osteoblasts to proliferate.

We found no significant influence of age on fusion rate. However, this might represent a type 2 error, due to the relatively low number of patients in the groups 'incomplete union' and 'non-union'.

There was no influence of sex and diagnosis on osteoblast proliferation. This is in accordance with previous clinical findings after posterolateral spinal fusion [2, 3, 17, 31, 38].

It is well known that loading stimulates osteogenesis, perhaps through an influence on the proliferative ability of osteoblasts. Other bone and osteoblast characteristics may also play a role in predicting successful fusion. Despite the large amount of knowledge gained from previous spinal fusion studies, the molecular biology of the fusion process is not yet fully understood. Further studies on gene expression in the early stage fusion mass will hopefully improve our understanding of osteoblast behavior, bone formation, and the influence of osteoinductive proteins at various stages of fusion.

Silcox et al. [29] have shown in an animal model that the presence of systemic nicotine favors the development of non-union. Riebel et al. [26] showed that nicotine decreases vascular ingrowth into autogenous cancellous bone graft in a rabbit model and that this vascular effect is reversible within 2 weeks of elimination of nicotine. They also found that the inhibitory effect of nicotine varies among animals, suggesting an individual predisposition. Daftari et al. [7] have shown in a model first reported by Deleu and Trueta [8], where cancellous bone is transplanted upon the iris, that nicotine inhibits revascularization of a cancellous bone graft. Our observation that preoperative tobacco consumption did not affect the in vitro osteoblast proliferation supports the hypothesis that nicotine exerts its effect through inhibition of angiogenesis in the fusion mass rather than through an effect on osteoblasts. In clinical practice, the most common approach to the problem of pseudarthrosis has been to control the mechanical environment. Biological manipulation of bone formation by various bone graft materials and graft substitutes [5, 6, 23] will perhaps provide better solutions in the future. However, improved understanding of osteoblast proliferation capacity may prove to be of value in the selection of those patients who would benefit from biological enhancement of the bone fusion capacity.

Further studies in larger patient populations and refinement of in vitro autologous osteoblast culturing are needed before any definitive conclusions can be drawn on the predictive value of this new assay with respect to bone healing in spinal fusion. However, with more sensitive testing and better discrimination, other results are possible or can in any event not be excluded.

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