DEVELOPMENT OF THE SPINE FOLLOWING PINEALECTOMY IN GROWING RATS

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Abstract


Idiopathic scoliosis is the most frequent structural spine deformity in its frontal plane. This disorder originates and gradually develops during the period of growth of children with initially straight spine. Three types of idiopathic scoliosis can be distinguished according to the onset of the first signs of spine curving: 1) infantile scoliosis encountered during the first three years of life, 2) juvenile scoliosis from three years of age to puberty and 3) adolescent idiopathic scoliosis from the beginning of puberty to growth cessation.

The whole spine except the neck may be affected. Great attention is at present paid both to the early diagnosis and therapy of this disorder due to its increased frequency of...
occurrence in the early youth of man. The diagnosis is based on a careful clinical and radiographic examination of the spine. On the basis of such results it is possible to make a prognosis and propose a precise plan of therapy. In growing individuals with a moderate deformity (from 25° to 40° according to Cobb) (Cobb 1948) conservative therapeutic methods are chosen. Many authors, however, proved that conservative therapy influences the development of scoliosis in a smaller number of patients than previously expected (Hopf et al. 1998; Chaloupka et al. 1992; Lonstein and Winter 1994; Nooman et al. 1996). There is indicated a surgical treatment consisting in the correction of the position of vertebrae with subsequent internal fixation in individuals with a marked spine deformity (from 40° to 50° according to Cobb).

The success of therapy is limited by the fact that the etiologies of idiopathic scoliosis remains unknown. Such animals as monkeys (Pincott et al. 1984), rabbits (Barrios et al. 1987; Carpierto et al. 1997; Pal 1991; Pal et al. 1991; Suk et al. 1989), rats (Barrios and Arrotegui 1992), and chickens (Coillard and Rivard 1996; Kanemura et al. 1997; Machida et al. 1993; Machida et al. 1996; Machida et al. 1994; Machida et al. 1997; Wang et al. 1997) were used for experiments aimed at the search for the etiology of scoliosis. From these experimental studies it is clear that idiopathic scoliosis is of multifactorial origin and development. There are several hypotheses concerning the etiology of idiopathic scoliosis described in the literature. Genetic (Archer and Dickson 1985; Goldberg et al. 1997; Keim 1976; Kesling and Reinker 1997; Le Blanc et al. 1997; Millner and Dickson 1996; Wynne-Davies et al. 1982), metabolic (Bradford et al. 1977; Worthington and Shambaugh 1993), hormonal (Bagnall et al. 1996; Coillard and Rivard 1996; Machida et al. 1996), skeletal (Grivas et al. 1991; Manganiello 1987; Millner and Dickson 1996; Sevastik et al. 1984; Tomaszewski 1989), myogenic (Karski 1996), neuromuscular (Keim 1976), and neurogenic (Barrack et al. 1988; Depoter et al. 1987; Herman et al. 1985; Keessen et al. 1992; Lewonowski et al. 1992; Machida et al. 1993; Machida et al. 1994; Maiocco et al. 1997; Mochida et al. 1993; O’Beirne et al. 1989; Roth 1996; Royo-Salvador 1996; Sahlstrand et al. 1979; Samuelsson et al. 1991; Wyatt et al. 1986; Zadeh et al. 1995) disorders are mentioned as main etiological factors. The importance of heredity was confirmed only in clinical cases and several experimental and clinical studies confirmed the influence of neurological dysfunctions localised in the central nervous system as well.

Apart from experiments on animals, computer modelling is used in the study of idiopathic scoliosis (Murray and Bulstrode 1996).

In the 1990’s the attention of scientists concentrated on the possibility of connection between the origin and development of idiopathic scoliosis and low amounts of serotonin which is produced in corpus pineale. Scientists performed surgical removal of corpus pineale in young chickens (Wang et al. 1997; Kanemura et al. 1997; Machida et al. 1994, 1996; Coillard and Rivard 1996) and their results supported the theory that serotonin plays a role in the development of idiopathic scoliosis.

The aim of this experimental study

Since experiments on chickens are of limited value in searching for the etiology of idiopathic scoliosis in man, it was our goal to study the influence of pinealectomy during body growth of some mammalian species on the development of spine.

Materials and Methods

Laboratory Norway rats (Rattus norvegicus v. alba f. domestica - Wistar albino rat) were selected as model animals. Ethics Committee Approval (September 19, 1997) was given to conduct the study according the standard protocol. The control group consisted of 8 healthy adult rats in which we determined the normal physiological spine curvature. Cervicothoracic lordosis and thoracolumbar kyphosis were measured.
Both males and females were included in the experiment. The pineal body was removed surgically in 24 rats at the age of 3 to 5 weeks when the individuals reached the mean body weight of 70 g (varying from 40 to 120 g). There was no medication administered to the rats after the surgical procedure and laterolateral and dorsoventral radiographs of spine were taken two times. The first radiographic examination was performed 8 weeks and the second 16 weeks after pinealectomy. Using radiographs taken 16 weeks after the surgery we measured the curves of cervicothoracic lordosis and thoracolumbar kyphosis and according to methods by Cobb (1948) we determined the degree of prospective scoliosis. Scoliosis cases over 8° were only considered to be clinically important (Barrios and Arrotegui 1992). For the purpose of radiographic examination the rats were anesthetized using halothane in an inhalation chamber and after the second radiography they were euthanized. Then their brain was removed, fixed by 10% formaldehyde and evaluated by an independent histopathologist to confirm the pineal body removal.

Surgical procedures were performed under intraperitoneal anesthesia (diazepam at the dose of 15 mg/kg in combination with 5% ketamine 60 mg/kg). Dorsocaudal approach to the pineal body during the surgery of the first four rats was used. At first, skin was incised in length about 1.5 cm in the median line of the dorsocaudal cranium right after confluens sinuum and then a circular opening of about 3 mm diameter in the cranium with help of a special burr under continuous cooling by saline solution was made. Dura mater was perforated in the length of 1-2 mm using a hypodermic needle of 0.7 mm diameter. After that, the pineal body was removed using anatomical tweezers. Reliability of this surgical procedure was verified before the start of the experiment by histopathological examination of brains from three cadavers of rats. During the surgical procedure we encountered massive bleeding in three rats and had to take immediately quite exacting steps for resuscitation. Because of these problems we changed the surgical procedure. The modification consisted in preparation of the subcutis of the caudodorsal area of cranium from the left and cranial opening in the angle between sinus sagittalis and sinus transversus on the left. Bleeding was minimised and due to proper cooling of the bone burr during cutting of the cranium there was no need for resuscitation. The surgical wound was only closed by simple interrupted suture of the skin using non-absorbable monofilament material. Recovery from anesthesia was quick, mostly within 30 minutes, and without motor dysfunctions.

Statistical significance of our results was evaluated using Fisher’s test (relationship between the development of scoliosis and pinealectomy) and Mann-Whitney U-test (differences in the angle of scoliosis of experimental and control rats). The curves of cervicothoracic lordosis and thoracolumbar kyphosis in both groups were evaluated by Student’s t-test. Statistical analyses were done using Stat plus 1.10 (Matoušková et al. 1992).

Results

Pinealectomy was completed successfully in all 24 rats. It was not necessary to administer any analgesic agents or antibiotics to the rats. There were no healing complications of surgical wounds. The intraperitoneal anesthesia (diazepam in the dose of 15 mg/kg in combination with 5% ketamine in the dose of 60 mg/kg) proved to be quite adequate for this surgical procedure. Time needed for the onset of surgical stage of anesthesia (from the intraperitoneal injection to the start of operation) was 5 to 11 minutes, i.e. 7 minutes on average. The surgical procedure lasted 5 to 30 minutes (it was 30 minutes at the beginning of this experiment, and then it gradually took less time) with the average of 13.5 minutes. There was no need to prolong the anesthesia by further administration of anesthetics during the procedure.

During the first radiographic examination two rats died due to halothane inhalation anesthesia. Therefore, only 22 individuals (15 males and 7 females) could be used for the final evaluation of the study. Radiographs taken 8 weeks after the surgery served only as control of the spine development and were not used for any measurements. It was the second radiographic examination 16 weeks after the surgery that was used to evaluate the development of scoliosis. The rats were euthanized after this examination. At that time, their body weight amounted to 425 g on average (250 – 600 g), with males being considerably larger than females. We found much quicker somatic and sexual maturation in the experimental group as compared to controls. This fact enabled active reproduction in two females. The mean body weight of laboratory Norway rats described in literature is 400 g.

The cervicothoracic lordosis measured using the Cobb’s method from the second to the seventh cervical vertebrae in pinealectomised rats was -26° to -48°, i.e. -40° on average. The mean value of the thoracolumbar kyphosis in pinealectomised rats, measured from the seventh thoracic vertebra to the cranial border of the sacral bone, was +25°. Individual values varied from +16° to +33°.
Values of the cervicothoracic lordosis in the control group of 8 healthy adult individuals, measured before the experiment, varied from -39° to -63°, with the average of -48.3°. The thoracolumbar kyphosis in the control group was equal to +41° to +54°, with the average of +48°. There was 0° scoliosis found in all 8 control rats.

The mean scoliosis in the group of all 22 pinealectomised rats amounted to 5.4°. Such scoliosis cases in which we found values from 9° to 14°, i.e. 10.8° on average, were clinically important. They were found in 5 rats (4 males and 1 female), i.e. in 22.7% of all cases. The curvature aimed to the left (Plate XII, Fig. 1) in four cases. The apex of the curves in left-hand deviations was situated once between T4 and T5, once between T12 and T13, and twice between L3 and L4. In the case of right-hand curvature it was between T10 and T11. The curvatures were heterogeneous, of different length and with apices in various parts of the spine. All the curvatures were non-structural and without rotation of vertebrae.

The excision of corpus pineale was confirmed by an independent histopathologist after euthanasia of experimental rats.

There are surveyed the indices monitored during the experiment in the group of 17 individuals without scoliosis after pinealectomy and in the group of 5 rats with measured scoliosis in Tables 1 and 2, respectively.

<table>
<thead>
<tr>
<th>No</th>
<th>Sex</th>
<th>g</th>
<th>Time min.</th>
<th>x-ray curv.</th>
<th>x-ray curv.</th>
<th>C2</th>
<th>T7</th>
<th>S1</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (2)</td>
<td>M</td>
<td>40</td>
<td>30</td>
<td>0°</td>
<td>0°</td>
<td>-35°</td>
<td>+22°</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td>2. (4)</td>
<td>F</td>
<td>50</td>
<td>22</td>
<td>T13 5° L6</td>
<td>dx</td>
<td>0°</td>
<td>-41°</td>
<td>+28°</td>
<td>250</td>
</tr>
<tr>
<td>3. (7)</td>
<td>M</td>
<td>70</td>
<td>20</td>
<td>T3 4° L2</td>
<td>Sm</td>
<td>T3 6° L2</td>
<td>Sm</td>
<td>-30°</td>
<td>+28°</td>
</tr>
<tr>
<td>4. (8)</td>
<td>M</td>
<td>70</td>
<td>15</td>
<td>T11 6° L6</td>
<td>Sm</td>
<td>0°</td>
<td>-37°</td>
<td>+25°</td>
<td>600</td>
</tr>
<tr>
<td>5. (10)</td>
<td>M</td>
<td>60</td>
<td>15</td>
<td>T11 19° L6</td>
<td>Sm</td>
<td>T12 6° L6</td>
<td>Sm</td>
<td>-48°</td>
<td>+25°</td>
</tr>
<tr>
<td>6. (13)</td>
<td>M</td>
<td>70</td>
<td>13</td>
<td>T13 5° L6</td>
<td>Dx</td>
<td>T9 4° L6</td>
<td>Sm</td>
<td>-35°</td>
<td>+27°</td>
</tr>
<tr>
<td>7. (14)</td>
<td>F</td>
<td>70</td>
<td>10</td>
<td>0°</td>
<td>T5 4° L2</td>
<td>Sm</td>
<td>-38°</td>
<td>+28°</td>
<td>350</td>
</tr>
<tr>
<td>8. (15)</td>
<td>M</td>
<td>80</td>
<td>16</td>
<td>T9 4° L6</td>
<td>Sin</td>
<td>T9 8° L6</td>
<td>Sin</td>
<td>-33°</td>
<td>+19°</td>
</tr>
<tr>
<td>10. (19)</td>
<td>M</td>
<td>90</td>
<td>16</td>
<td>0°</td>
<td>0°</td>
<td>-26°</td>
<td>+16°</td>
<td>450</td>
<td></td>
</tr>
<tr>
<td>11. (21)</td>
<td>F</td>
<td>90</td>
<td>13</td>
<td>0°</td>
<td>L1 7° L6</td>
<td>Dx</td>
<td>-48°</td>
<td>+19°</td>
<td>350</td>
</tr>
<tr>
<td>12. (22)</td>
<td>M</td>
<td>120</td>
<td>15</td>
<td>0°</td>
<td>T10 6° L6</td>
<td>Dx</td>
<td>-48°</td>
<td>+27°</td>
<td>400</td>
</tr>
<tr>
<td>13. (23)</td>
<td>M</td>
<td>120</td>
<td>15</td>
<td>C6 6° T10</td>
<td>Sin</td>
<td>C6 3° T10</td>
<td>Dx</td>
<td>-41°</td>
<td>+21°</td>
</tr>
<tr>
<td>14. (25)</td>
<td>M</td>
<td>60</td>
<td>8</td>
<td>T3 3° T12</td>
<td>Dx</td>
<td>T7 5° L2</td>
<td>Sm</td>
<td>-42°</td>
<td>+33°</td>
</tr>
<tr>
<td>15. (26)</td>
<td>M</td>
<td>60</td>
<td>9</td>
<td>0°</td>
<td>0°</td>
<td>-41°</td>
<td>+31°</td>
<td>450</td>
<td></td>
</tr>
<tr>
<td>16. (30)</td>
<td>F</td>
<td>55</td>
<td>6</td>
<td>T13 4° L6</td>
<td>Dx</td>
<td>C6 1° T13</td>
<td>Dx</td>
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<td>+18°</td>
</tr>
<tr>
<td>17. (32)</td>
<td>F</td>
<td>50</td>
<td>8</td>
<td>T7 6° L1</td>
<td>Sin</td>
<td>T10 4° L6</td>
<td>Dx</td>
<td>-36°</td>
<td>+20°</td>
</tr>
</tbody>
</table>

Legend:
1st column: rank number, experimental individual’s number in parentheses
2nd column: sex (M-male, F-female)
3rd column: body weight at the time of operation in grams
4th column: duration of the operation in minutes
5th column: caudoventral radiographic view 2 months after the operation, in degrees according to Cobb (T-thoracic, L-lumbar, number of the vertebrae)
6th column: convexity of the curvature (dx-right hand, sin-left hand)
7th column: caudoventral radiographic view 4 months after the operation, in degrees according to Cobb (see the 5th column)
8th column: convexity of the curvature (see the 6th column)
9th column: laterolateral radiograph of the spine in degrees according to Cobb (terminal vertebrae are C2-i.e. the second cervical one, T7-i.e. the seventh thoracic one, S1-i.e. the cranial border of the sacral bone). The sign minus indicates lordosis, plus kyphosis
10th column: body weight in grams four month after the operation
No scoliosis case was found in the group of 8 control healthy adult animals. There were 5 cases of scoliosis found (22.7%) in the group of 22 evaluated rats which were subjected to the surgical procedure. Evaluating the dependence of scoliosis on pinealectomy by the Fisher’s test there were no significant findings on the 5% level. Because the number of cases was low, it is necessary to examine the occurrence of scoliosis in a much more numerous group of experimental rats. Using the Mann-Whitney U-test to evaluate the differences in the angle measured according to Cobb (0° in the control group and 5.4° in the experimental one, on average) we found significant differences (p < 0.01). Significant differences between both groups were also found in curves of the thoracolumbar kyphosis (p < 0.01) by the Student’s t-test. As far as the cervicothoracic lordosis is concerned, the t-test was not significant. Interpreting such results we have to bear in mind high variability found in this index.

### Discussion

When selecting a suitable animal species for our experimental study, it was necessary to consider its phylogenetic classification, duration of somatic maturation and demands of its breeding. After considering the above-mentioned aspects, laboratory Norway rats belonging to the mammalian class were chosen. They mature quickly, are not so demanding on the conditions of breeding and are very hardy. What is disadvantageous is the fact that they, contrary to man, do not move in an upright position, but are quadrupedal. The age of rats used for the experiment was chosen with regards to the requirement of performing pinealectomy as soon after the birth as possible in order to study its influence on the development of the animals.

<table>
<thead>
<tr>
<th>No</th>
<th>Sex</th>
<th>g</th>
<th>Time</th>
<th>x-ray</th>
<th>curv.</th>
<th>x-ray</th>
<th>curv.</th>
<th>C2</th>
<th>T7</th>
<th>S1</th>
<th>g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>(18)</td>
<td>M</td>
<td>70</td>
<td>15</td>
<td>T1</td>
<td>10°</td>
<td>L2</td>
<td>Dx</td>
<td>L1</td>
<td>L6</td>
<td>14°</td>
</tr>
<tr>
<td>2.</td>
<td>(20)</td>
<td>M</td>
<td>90</td>
<td>15</td>
<td>T13</td>
<td>3°</td>
<td>L6</td>
<td>Sin</td>
<td>T6</td>
<td>L6</td>
<td>10°</td>
</tr>
<tr>
<td>3.</td>
<td>(24)</td>
<td>M</td>
<td>60</td>
<td>12</td>
<td>0°</td>
<td>L1</td>
<td>L6</td>
<td>10°</td>
<td>Sin</td>
<td>-43° +31°</td>
<td>450</td>
</tr>
<tr>
<td>4.</td>
<td>(28)</td>
<td>F</td>
<td>50</td>
<td>7</td>
<td>T10</td>
<td>5°</td>
<td>L6</td>
<td>Sin</td>
<td>C6</td>
<td>T10</td>
<td>11°</td>
</tr>
<tr>
<td>5.</td>
<td>(29)</td>
<td>M</td>
<td>50</td>
<td>5</td>
<td>T2</td>
<td>3°</td>
<td>T12</td>
<td>Dx</td>
<td>T2</td>
<td>L6</td>
<td>9°</td>
</tr>
</tbody>
</table>

Legend:
1st column: rank number, experimental individual’s number in parentheses
2nd column: sex (M-male, F-female)
3rd column: body weight at the time of operation in grams
4th column: duration of the operation in minutes
5th column: caudoventral radiographic view 2 months after the operation, in degrees according to Cobb (T-thoracic, L-lumbar, number of the vertebra)
6th column: convexity of the curvature (dx-right hand, sin-left hand)
7th column: caudoventral radiographic view 4 months after the operation, in degrees according to Cobb (see the 5th column)
8th column: convexity of the curvature (see the 6th column)
9th column: laterolateral radiograph of the spine in degrees according to Cobb (terminal vertebrae are C2-i.e. the second cervical one, T7-i.e. the seventh thoracic one, S1-i.e. the cranial border of the sacral bone). The sign minus indicates lordosis, plus kyphosis
10th column: body weight in grams four month after the operation
have not been published more than one experimental study devoted to the development of scoliosis in rats after surgical removal of the pineal body (Dubousset et al. 1983) as yet.

The kind of anesthesia and the surgical procedure were selected on the basis of compilation of published data and our own experiences obtained during preparation for the experiment using control animals. The anesthesia chosen was easy to perform, inexpensive, quickly induced and with its length and depth of sufficient quality.

The animals were kept under conditions that correspond with species requirements. The radiographic examination in 8 weeks after the surgery, i.e. during growth, was done only for the purpose of control evaluation of spine development. For definitive diagnosis and scoliosis measurements to be done, it was necessary to evaluate radiographs taken 16 weeks after the procedure, i.e. after the end of growth in the experimental rats. Selection of the surgical procedure (pinealectomy) was based on following considerations. Affects in the muscles, nervous radices, vertebrae or ribs lead to the development of scoliosis of various degree. There were not, however, found primary defects in these tissues causing scoliosis. Injuries of the central nervous system on various levels lead also to the development of scoliosis. The development of scoliosis was best described in chickens after pinealectomy (Coillard and Rivard 1996; Kanemura et al. 1997; Machida et al. 1993; Machida et al. 1996; Machida et al. 1994; Wang et al. 1997). In chickens it is possible to explain the development of scoliosis by extraordinary numerous melatonin receptors (in the brain stem, gray matter of the spinal cord, especially in the sacral area) (Wan and Pang 1994).

In other animal species these receptors are found in limited numbers in thalamus, hypothalamus and hypophysis (Fang et al. 1990; Laitinen et al. 1990; Laitinen and Saavedra 1990; Utiger 1992). Fagan et al. (1998) on the basis of their study of melatonin levels in stable and progressive idiopathic scolioses excluded the possibility of their therapy using melatonin. The melatonin model describing the origin of scoliosis in chickens, in some author’s opinion (Machida et al. 1993; Machida et al. 1994; Machida et al. 1997), may aid to discover similar receptors influencing decompensation of postural functions which lead to the development of scoliosis in man. Contrary to this opinion, negligible importance of experimental studies using chickens in the search for the etiology of human idiopathic scoliosis is witnessed by different structure of the brain cortex in addition to the above-mentioned facts concerning melatonin receptors and different numbers of vertebrae (Coillard and Rivard 1996).

Only a few studies on the influence of pinealectomy were done using other animal species. Stebbins (1960) did not find scoliosis in lizards and the same conclusion was obtained by Dubousset et al. (1983) in the only study which concerned this surgical procedure in rats. Only a moderate scoliosis (9° to 14°) developed in a small number of our experimental rats (22.7%). We encountered, however, peroperative bleeding which might be causing the development of scoliosis.

The mean body weight of adult rats mentioned in published sources is equal to 400 g. Females are smaller and of lower weight than males. In our experiment we found quicker maturation and higher mean body weight in pinealectomised rats. This fact has not been described in scientific papers yet. It may be explained by impairing hormonal functions and the so-called biological clock.

In healthy adult rats we measured the cervicothoracic lordosis in values from -39° to -63°, i.e. -48.3° on average; and the thoracolumbar kyphosis from +41° to +54°, i.e. +48.0° on average (our own findings). The cervicothoracic lordosis in pinealectomised rats amounted to –26° to –48°, i.e. –40° on average. The mean thoracolumbar kyphosis in pinealectomised rats was equal to +25°. The individual values varied from +16° to +33°.

Evaluating our experimental results we have to consider the possibility that other factors, such as injuries to the surrounding brain tissue during the procedure (Chaloupka 1998),
brain hypoxia, and metabolic-toxic effects of anesthetics, might have participated in the development of scoliosis. On the other hand, in experimental individuals without scoliosis development we may suppose compensatory mechanisms outweighing the development of spinal changes.

Vliv odstranění corpus pineale na vývoj páteře u rostoucích potkanů


U skupiny 22 potkanů po odstranění corpus pineale ve věku 3 až 5 týdnů autoři zaznamenali rychlejší růst. Průměrná hmotnost za 4 měsíce dosáhla 425 g. Cervikotorakální lordóza (C2 – T7) měřila průměrně -40°, torakolumbální kyfóza (mezi T7 a S1) měřila v průměru +25°. Skolióza byla zjištěna u 5 potkanů (4 samců), tj. ve 22,7% případů. Ctyřikrát šlo o křívu levostrannou s vrcholem mezi T4 – T5, T12 – T13, a dvakrát mezi L3 – L4. Skolióza měla v průměru 10,8° (9° až 14°). V kontrolní skupině 8 zdavých dospělých potkanů jsme nezjistili žádného jedince se skoliózou. Ve skupině 22 operovaných potkanů jsme zjistili skoliózu v 5 případech (22,7%). Při hodnocení závislosti výskytu skoliózy na pinealektomii Fisherovým testem jsme neprokázali závislost na 5% hladině významnosti. Statisticky významné rozdíly mezi oběma skupinami jsme zjistili v křívkách torakolumbální kyfózy (p<0,01) Studentovým t-testem. V případě cervikotorakální lordózy byl t-test neprůkazný.

Pinealektomie spolu s lokalizovanou ischémií a peroperačním krvácením mohou být potkány příčinou skoliózy. Tyto faktory mohou ovlivnit výskytu torakolumbální kyfózy.

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Fig. 1: Rat No. 18. Laterolateral (cervicothoracic lordosis of $-45^\circ$ between C2 and T7 and thoracolumbar kyphosis $+30^\circ$ between T7 and S1) and dorsoventral (left-hand scoliosis of $14^\circ$ between L1 and L6) radiographs 4 weeks after the surgical procedure.