Acta Orthop. Belg., 2006, **72**, 247-260

CURRENT CONCEPTS REVIEW

Pathogenesis of progressive adolescent idiopathic scoliosis Platelet activation and vascular biology in immature vertebrae : an alternative molecular hypothesis

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Altered paraspinal muscle activity was suggested by Lowe *et al* (2002) to explain a relationship between Cobb angle changes and platelet calmodulin level changes in adolescent idiopathic scoliosis (AIS). We formulate an alternative *platelet-skeletal hypothesis* which involves : (1) a small scoliosis curve ; (2) axial loads transmitted directly from the intervertebral discs to vertebral body growth plates (endplate physes) as axial inward bulges that create mechanical micro-insults ; (3) the latter cause dilatation of juxta-physeal vessels and, in deforming vertebrae, vascular damage with exposure of subendothelial collagen and other agonist proteins ; (4) subject to predisposition, platelet activation with calmodulin changes occurs within dilated vessels of deforming vertebral bodies ; (5) the activated platelets in juxta-physeal vessels release growth factors that, after extravasation, abet the hormone-driven growth of the already mechanically-compromised vertebral endplate physes to promote the relative anterior spinal overgrowth and curve progression of AIS. The hypothesis links several fields in each of which research within ethical restraints is suggested to refute it.

Keywords : scoliosis ; idiopathic ; aetiology ; platelets ; calmodulin ; growth plate.

INTRODUCTION

The possible relation of platelets to adolescent idiopathic scoliosis (AIS) pathogenesis has been explored by several workers in the last 20 years. In the early 1980s, predicated on knowledge that platelets and muscle fibres share the same contractile proteins (actin & myosin), abnormalities in the structure, function and biochemistry of platelets were reported in patients with AIS ; these platelet changes were attributed to a defect of calcium transport in membrane and/or contractile protein metabolism (*39, 69, 127, 161*). Although many platelet defects were identified most have not been confirmed (*36, 63, 140, 144*).

Lowe *et al* (*72*) re-examined the problem using a new method that involves the activation of a calcium receptor protein – calmodulin (*65, 72, 73*). In a longitudinal study of 55 patients (girls 51, boys 4) with AIS of various types, they found a relationship

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No benefits or funds were received in support of this study

between platelet calmodulin levels and scoliosis curve changes that occurred either spontaneously, or in relation to brace treatment or surgery. Lowe *et al* (*72*) argued, as had Liebergall *et al* (*69*), that since platelets are not directly involved in the biomechanics of the spine the platelet activation was not a secondary result of the spinal curvature ; they supported the view of Yarom *et al* (*161*) that the platelet is a 'mini' skeletal muscle with a similar protein contractile system (actin and myosin) and suggested that platelet calmodulin acts as a systemic mediator for tissues with a contractile system (actin and myosin). Recently, a high prevalence of abnormal pre-operative coagulation tests has been reported in patients with adolescent idiopathic scoliosis (*53*) ; further sophisticated tests of platelet morphology and function were recommended (*136*).

An electronic debate of Lowe's research (*73*) led us to formulate an alternative *platelet-skeletal hypothesis* (*20*) with relevance to the pathogenesis of adolescent idiopathic scoliosis (*19, 48, 71, 108*). Preliminary reports of the hypothesis have been published (*20-22*).

STATEMENT OF THE PLATELET/SKELETAL HYPOTHESIS

The platelet/skeletal hypothesis involves morphological, mechanical, vascular, platelet, hormonal and growth mechanisms within the field of molecular orthopaedics (*35*). It has six requirements (figs 1-3).

1) A small scoliosis curve.
2) In the human upright position, repeated vertebral loading with mechanical forces created in the discs during activities of daily living distort the vertebral body growth plates (endplate physes) as repeated axial inward bulges creating microinsults.
3) These microinsults activate endothelial cells resulting in dilated vessels and vascular "lakes" (resembling bunches of grapes) adjacent to the disc growth plates of *normal* vertebrae from 9 to 13 years of age.
4) Platelets, as they circulate through vessels in eccentrically-loaded and deforming immature vertebral bodies particularly about the curve apex on the medullary aspect of endplate physes, are activated by a) the slowing of blood flow in the dilated vessels and vascular "lakes", b) repeated mechanical micro-insults that cause both shear stresses, and c) vascular damage with exposure of subendothelial collagen and other agonist proteins. Platelet activation is associated with the formation of a calcium-calmodulin complex.
5) Growth factors released from platelets after extravasation abet the hormone-driven growth of the already mechanically-compromised disc growth plates to promote relative anterior spinal overgrowth and promote curve progression
6) A molecular predisposition to platelet-activation. The possible tissues affected by the putative predisposition include platelets, endothelium and subendothelium. The molecular predisposition may : a) differ between girls and boys with progressive AIS and between subjects of the same sex ; b) involve hormones ; and c) include genetic causes including polymorphisms associated with the phenotypes of these tissues (*1, 13, 59, 76, 138, 159*).

A female perspective. The predilection of adolescent females for progressive AIS is part of the wider problem of female/male differences in disease manifestation and drug response the understanding of which is currently under intense scrutiny in relation to women's health (*87, 115*). The platelet-skeletal hypothesis deduces possible molecular mechanisms for evaluation about the female susceptibility to progressive AIS.

COMPONENTS OF THE HYPOTHESIS

The components of the platelet/skeletal hypothesis act sequentially like a cascade in the development of progressive AIS (fig 1).

1. Normal human immature vertebral bodies and vascular biology

Vertebral cartilage endplates bulge inwards under load. The lack of ossified epiphyses in human vertebral bodies (*42*) causes axial loads to be

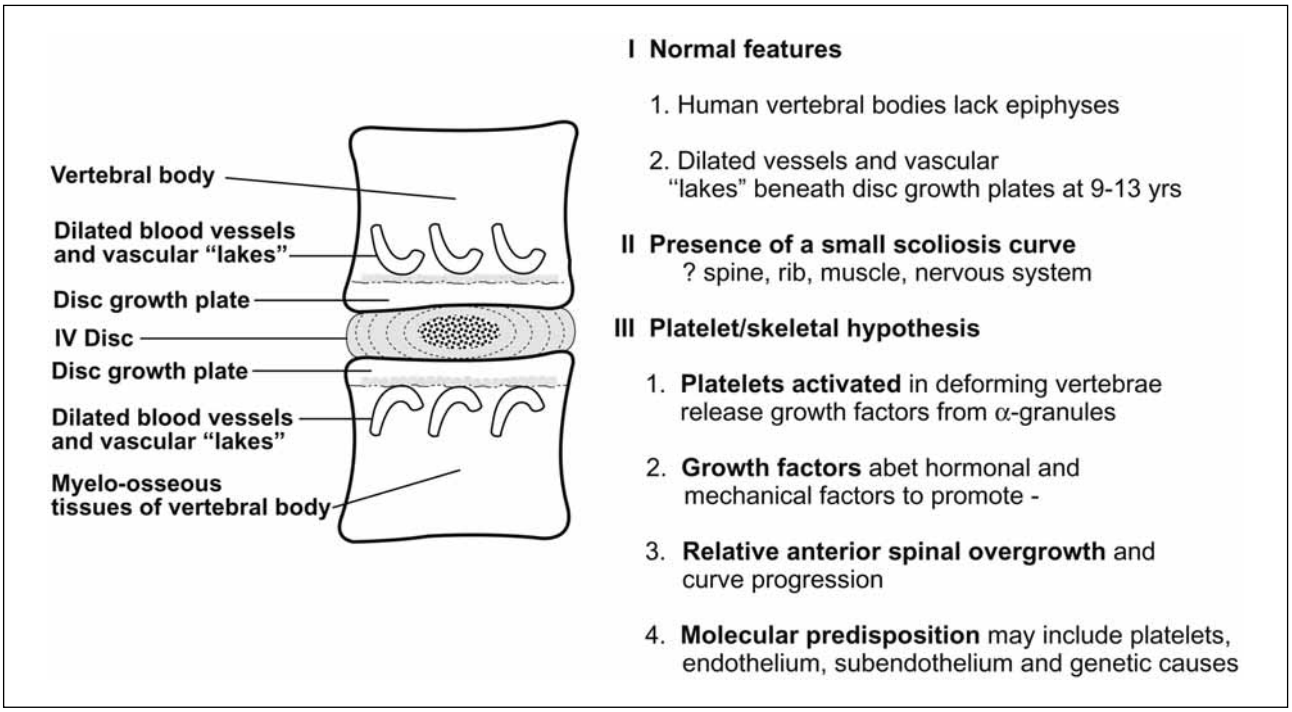

Fig. 1. – Diagram to show platelet/skeletal hypothesis for progressive AIS involving immature human vertebrae, intervertebral discs, growth plates, dilated blood vessels, vascular "lakes" and platelets.

transmitted from the intervertebral disc directly to vertebral body endplate physes (*50*) as axial inward bulges (*15*) (fig 2) ; in mature vertebrae others (*55*) describe this movement as small. The platelet/skeletal hypothesis states that repeated axial inward bulges of vertebral body physes create mechanical micro-insults causing shear stresses, dilatation of juxta-physeal vessels with vascular damage causing exposure of subendothelial collagen and other proteins of vertebral vessels ; such stresses are increased by disc degeneration (*32, 91*). Haemodynamic stress has a critical effect on the biology of cells in blood vessels (*43, 82, 114, 141*) ; the haemodynamic forces within blood vessels are resolved into two principal forces namely *a*) *shear stress* and *b*) *intra-vascular pressure* which the endothelium transduces into biological responses with nitric oxide as one of the pivotal molecules coordinating vascular function (*114*).

Dilated vessels and vascular "lakes" in normal vertebrae (*figs 1, 3*). Dilated vessels and vascular "lakes" were found by Mineiro (*89*) at each end of normal vertebral bodies but only from 9-13 years of age (fig 3). We postulate that these vessels dilate in response to the microinsults through the action of vasoactive molecules, and their dilatation induces platelet activation from both vascular stasis and shear stresses created by the micro-insults. In immature normal vertebrae Crock and his colleagues (*27-29*) describe fine arteries penetrating the cartilage caps in the form of *sinusoidal expansions* that disappear as the "ossification of the vertebral body extends near to the final vertebral end-plate zone". To our knowledge there are no reports of blood vessels supplying the disc growth plates of vertebrae, lumbar or thoracic, in AIS. Ratcliffe (*117*) speculates that AIS results from retarded growth on the affected side due to failure of the extra-osseous arterial supply to compensate for the normal reduction during childhood and adolescence of anastomoses between the arteries supplying the radial sectors of juxta-physeal regions of immature verte-

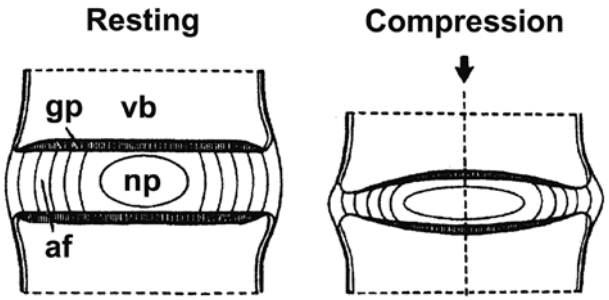

Fig. 2. – Schematic diagram showing the deformation of the intervertebral disc and growth plate under load : gp = disc growth plate, vb = vertebral body, af = annulus fibrosus, np = nucleus pulposus [modified from Roberts *et al* (*125*).

brae. In neurological scoliosis, angiography revealed abnormal vascular anatomy in three subjects and CT angiograms are recommended in such patients (*35*).

Molecular mechanisms of vascular biology – vasodilators and vasoconstrictors. The molecular mechanisms that dilate vessels of the vertebral body growth-plate cartiIages (*89*) are likely to involve a disturbances in the balance of local vasodilators – including *nitric oxide* (*68, 85, 86, 120*), *prostacyclin* (*68, 134, 138, 152*) *and anandamide* (*40, 54, 77*), and vasoconstrictors – including *endothelin-1* arising from activated endothelium and possibly platelets (*68, 152*). In endothelium, activation is fol-

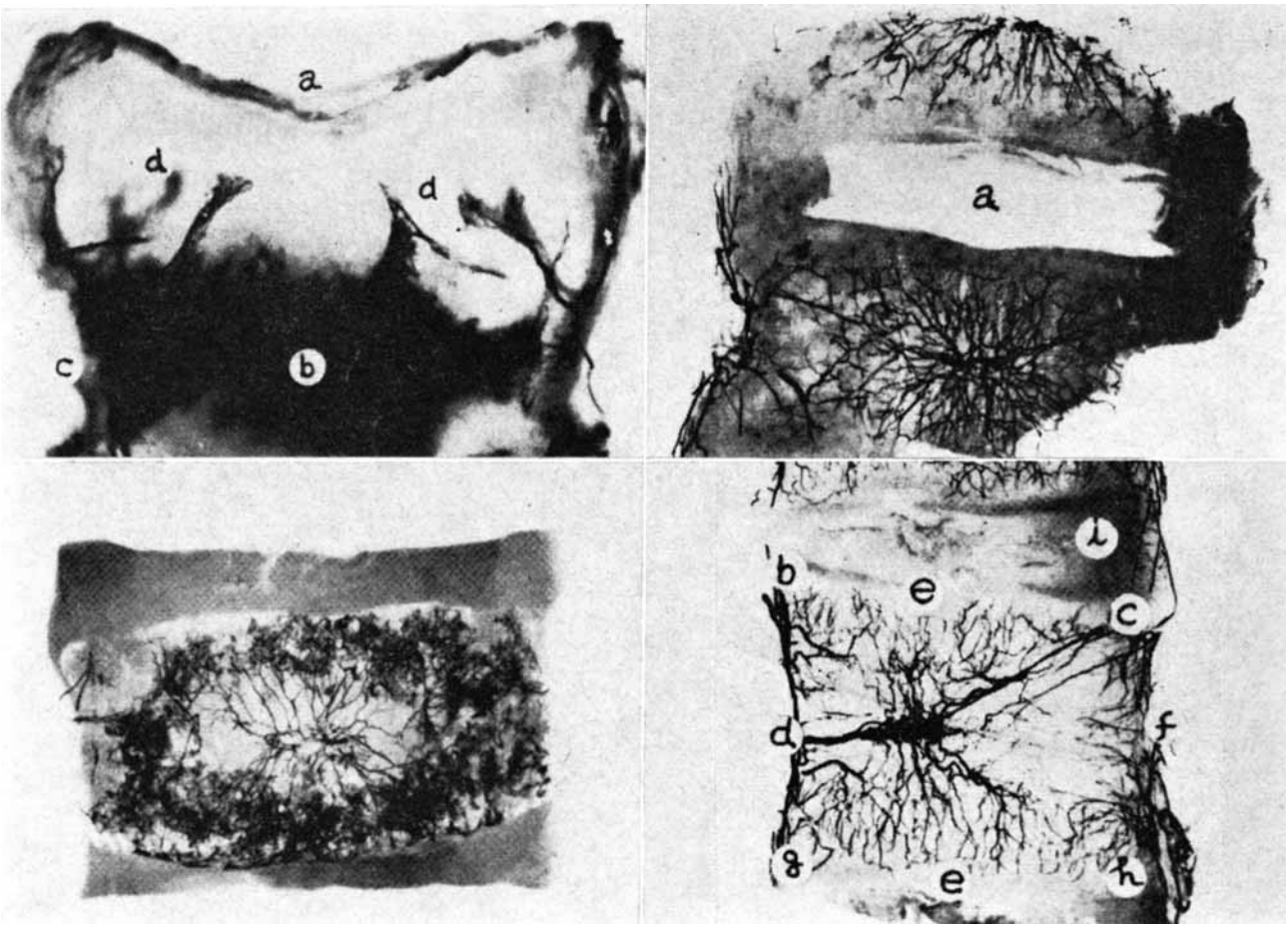

Fig. 3. – Longitudinal sections through normal human vertebrae after injection of the blood vessels. *Upper left,* a fetus of 6 months ; *upper right,* a child of 3 years ; *lower left,* a child of 10 years showing dilated vessels and vascular "lakes" ; and *lower right,* an adult of 25 years. For explanation see text. (Reproduced from Burwell (*18*) when modified from and by kind permission of the late-Dr J. D. Mineiro and the Faculty of Medicine, Lisbon, and Elsevier).

lowed in a few hours by changes in the regulation of a subset of genes, both upregulation (eg *nitric oxide synthase*) and downregulation (eg *endothelin-1*) (*43, 114*) affecting permeability (*86*). Estrogen causes vasodilatation through both rapid increase in nitric oxide production and induction of NO genes (*87*).

Nitric oxide (*NO*), a biologically active unstable radical and a member of the reactive nitrogen species, is an intercellular messenger molecule critical to numerous biological processes including vascular tone. It is removed by the antioxidant defence system (*49, 88, 132, 160*). Shear stress as well as dilating vessels exerts an antiproliferative effect on vascular walls (*82*) and causes permeability changes thought to increase exposure to oxygen radicals such as superoxide (*114*). In articular chondrocytes shear stress and fluid-induced shear stress increase *NO* release (*31, 67*). There is a need to evaluate vertebral body growth plate chondrocytes experimentally for release of *NO synthase* and *NO* during exposure to each of mechanical and fluid-induced shear stress (*7, 30, 31, 67*).

Physeal loading, exposure of collagen and other proteins. In deforming vertebrae, removal of endothelium exposes platelets to numerous subendothelial adhesive proteins including collagen (*26*) and the von Willebrand factor (*70*). In this connection repeated mechanical factors applied to the hands (vibration) and feet (jumping) are thought to cause desquamation of vascular endothelial cells, blood cell damage, and the development of disordered or eddy flows in the blood stream (*142*). During physeal loading the role of fluid-induced shear stress on local vessels caused by fluid exuded from discs through the cartilage endplates is unclear (*7, 30*).

Marker proteins of endothelial cell activation, vibration, physical exercise and AIS. The von Willebrand factor (*vWF*) is a complex plasma protein whose release is a marker for endothelial damage (*70, 112, 151*). There is evidence that vibration has a significant effect in increasing back discomfort and the serum levels of *vWF* antigen possibly arising from vibration-induced vascular damage within the spine (*112*). Additional markers in the plasma of endothelial dysfunction include *vWF*-propeptide, tissue-type plasminogen activator (*tPA*), endothelin-1 (*ET-1*) and the stable end product of nitric oxide (*NO*), nitrite/nitrate (NO_x) (*80, 142, 150, 154*). The variability in *vWF* plasma levels is substantial among normal subjects and the *vWF*-propeptide is preferred to *vWF* as a marker of endothelial secretion (*154*). The above factors need evaluating in relation to AIS ; changes in both fibrinolytic activity and *tPA* might be useful in assessing the vascular effects of local mechanical stimulation of the spine during physical exercise (*80, 142*) and/or exposure to vibration (*142*). But any such changes may be restricted to the local venous blood from the spine and not detected systemically due to dilution.

Radiological studies suggested in AIS. Dilated vessels and vascular "lakes" in the living subject may be sought by using gadolinium-enhanced MR imaging (*61*) and specifically a serial MRI study of diffusion characteristics in the spine after gadodiamide injection (*116*). Any positive radiological findings in combination with the detection of a) platelet dysfunction, and/or b) endothelial-derived marker plasma proteins in response to physical exercise and/or exposure to vibration may enable the discrimination of progressive from nonprogressive scoliosis curves.

Vertebral body marrow and endplate changes in adults with degenerate discs. MR imaging of adults with degenerative disc disease and low back pain has revealed a spectrum of vertebral body marrow changes adjacent to the end plates (*32, 91*) ; the changes are attributed to the degenerative process resulting in greater axial loading and increased stress on the vertebral body endplates (*91*). The vertebral endplate abnormalities of the Modic changes on MRI are attributed to inflammation and axon ingrowth induced by tumor necrosis factor (*105*).

2. Platelet calmodulin and secretion of growth factors by activated platelets

S. Heptinstall (personal communication) writes : *Platelet calmodulin is not usually used as a marker of platelet activation in the thrombosis field. The platelet/skeletal hypothesis for progressive AIS suggests evaluating the haemostatic system further*

including two platelet-secreted proteins, β-thromboglobulin (βTG) and platelet factor 4 (PF4) both classic α-granule markers, to detect activation of circulating platelets. Soluble P-selectin, expressed on the surface of activated platelets and platelet/leukocyte conjugates could also be evaluated as a measure of platelet activation. In addition some standard platelet aggregometry could be done to look for evidence of platelet exhaustion as an indicator of previous activation.

Platelet activation. Platelet activation is associated with elevated cytosolic Ca^{2+} derived from the dense tubular system by the opening of calcium channels (*51, 58, 97, 107, 118*) involving G proteins in the signalling processing (*158*). Rapid structural and chemical changes that activate platelet adhesion receptors, remodel the cell cytoskeleton with actin assembly to allow cell spreading and aggregation (*99*) ; the composition of the plasma membrane changes, and lead to the secretion and synthesis in granules of platelet activating factors and growth factors including those that affect angiogenesis. The release of α-granule glycoproteins and proteins is an extremely sensitive indicator of platelet activation, since they are released at lower concentrations of stimuli (e.g. thrombin) than that required for dense granules (*90, 96, 137*). The platelet release reaction is considered to be an important biological event that may be involved in many different pathological situations (*121*).

α-granules of platelets, growth factors and repair. In addition to their role in haemostasis the function of α-granules during platelet activation in the context of wounding is to release 'repairing factors' into the local tissue environment (*109, 118 121, 122*). Platelets may be the first source of growth factors initiating repair in both fractures (*3, 11, 41*) and bone grafts (*74, 81*). *Platelet growth factors* are currently being evaluated for their therapeutic potential in bone grafts (*4, 46, 81*), mechanisms of bone repair (*128*), and tendon repair (*5*). A commercial platelet separation system to procure autologous platelets to enhance repair is available (*Biomet Merck Biomaterials Gmbh,* D-64271 Darmstadt, Germany).

Platelets - a role in growth plate physiology and repair ? A possible role for platelets in growth plate physiology and/or repair has not been tested and needs experimental evaluation. In any such evaluation of mechanical stress on vertebral body growth plates, animals that lack vertebral body epiphyses – such as some insectivores including European hedgehogs and some shrews (*18, 139*) – should be used.

Dense granules in platelets. In AIS it has been stated that the identity of the electron-opaque granules in activated platelets is uncertain, and may correspond either to the 'dense bodies', or to the α-granules (*69*). The fewer *dense* (*δ*) *granules* function primarily to recruit additional platelets to sites of injury (*51, 84, 96, 118, 131*).

3. Growth factors in platelets, bone and growth plates

The α-granules are the largest and most abundant granules in platelets (*51, 118, 121, 122*) and besides proteoglycans (for relative stability) contain many different types of large proteins, such as adhesive proteins, coagulation factors and cellular mitogens (*51, 122*). The stored mitogenic growth factors include (*16, 25, 51, 52, 58, 109, 118, 121, 122*) :

- platelet-derived growth factor [a major mitogen (*52*)],
- TGF-β-1 & β-2 [the effects regulated by activation from a latent form (*124*)],
- basic fibroblast growth factor,
- platelet-derived epidermal growth factor,
- insulin-like growth factor-1, and
- connective tissue growth factor (*25*).

The *angiogenic regulators within platelets* are pro-angiogenic and anti-angiogenic (*16, 109*). The platelet/skeletal hypothesis requires that any growth factors secreted into medullary blood vessels supplying the disc growth plate gain access to the interstitial fluids bathing the vertebral body physes. This process by paracellular and transcellular pathways (*86*) would be facilitated by the dilatation of vessels as "lakes" thereby creating wide gaps between endothelial cells for the escape of molecules (*68*).

Platelet-derived growth factor (*PDGF*). PDGF has been detected in the mouse tibial growth

plate (*41*) but we are not aware of any studies of PDGF in human physeal material. PDGF is produced by osteoblasts as well as platelets and is mitogenic for osteoblastic cells *in vitro* with a probable role in both fracture repair (*3, 11, 23, 41, 46, 128*) and skeletal development (*56*) where it may exert autocrine and paracrine effects on osteogenesis. The platelet/skeletal hypothesis suggests a role for PDGF in AIS curve progression by stimulating relative anterior spinal overgrowth. Gruber *et al* (*46*) speculate that in bone repair PDGF is mitogenic with other factors of the BMP superfamily, including TGF-β, initiating the differentiation of skeletal cells.

Transforming growth factor-β (*TGF-β*) *in bone and platelets.* Bone represents the most abundant source of TGF-β in the body but the concentration of TGF-βs in bone is second to that of platelets (*6, 145*). TGF-β is a superfamily of cytokines with many functions that include enhancing the formation of matrix constituents (*66*). They function as an important regulators of endochondral ossification, bone repair and maintenance, may stimulate or inhibit (*2, 124, 155*), and act as a potent inducer of angiogenesis and fibrogenesis (*146*). In autopsy intervertebral disc material, TGF-βs were detected by two groups (*101, 146*), but not by a third group of workers (*135*). TGF-β, initially isolated from human blood platelets (*44*), has been localized in the human *neonatal rib* growth plate (*57*) -

- TGF-β2 was detected in all zones of the growth plate.
- TGF-β1 & TGF-β2 were highest in the proliferative and hypertrophic zone chondrocytes.

We are not aware of any study of TGF-βs in the vertebral disc growth plates of normal immature humans or AIS subjects, the need for which is suggested by the platelet/skeletal hypothesis. The viability of chondrocytes is reported to be more vigorous in the anterior spinal column than in the posterior spinal column of AIS patients (*162*). Calcification is frequently found in cartilage endplates excised at surgery (*125, 126*).

A role for skeletal growth factors in AIS pathogenesis ? Data are needed on growth factors in immature vertebrae in health and deformity before any potential role for them in the pathogenesis of progressive AIS can be evaluated. AIS curves that look radiologically similar may be very diverse at the molecular and genetic levels ; an attempt is being made to produce a functional and molecular classification of AIS (*95*).

4. Hormones, estrogen receptors, calmodulin, melatonin and AIS

Sex steroid hormones (*SSHs*) *and AIS.* Several authors have studied the hormonal control of skeletal growth in AIS but the implications of this research are presently unclear (*119*). The SSH estrogen is a candidate for curve progression (*59, 159*).

Estrogen, estrogen receptors and skeletal growth. According to Grumbach (*47*) and Savendahl (*130*) estrogen (largely estradiol [E2] but also estrone) is the critical sex hormone in the adolescent growth spurt of girls and boys acting largely indirectly through the pituitary growth hormone/IGF-1 (previously termed somatomedin-C) axis (*60, 123*), but also directly on growth plates (*130*). Local production of E2 has been demonstrated in rat costal chondrocytes (*24*). E2 levels are significantly higher in prepubertal girls than boys but are similar at peak height velocity (*47, 87*). Estrogens manifect their action in cells that contain a specific high affinity nuclear receptor (*83*). Estrogen receptors (ERs) have not been evaluated in the human intervertebral disc growth plate, but in human growth plates of neonatal ribs (*12*) and lower limb bones (*34, 102, 103*) the two subtypes of the ER have been detected with no difference between the sexes – encoded by separate genes, ERα & ERβ. The hypertrophic chondrocyte and osteoblasts may be the main target for estrogen in the growth plate in childhood and adolescence (*34*). ERs exist in a plethora of forms (*111*). Osteoblasts in 8/14 AIS patients showed a change in the ratio of ERα/ERβ mRNA receptor ratio that may alter estrogen signalling pathways (*92*). Deletion of the ERα genes in female mice leads to unchanged axial skeletal growth but decreased appendicular skeletal growth which may result from reduced serum IGF-1 levels (*153*). Estradiol has recently been shown to have antagonistic effects in the response to

melatonin of osteoblasts from 10 AIS subjects ; this is interpreted as a triggering factor in the pathogenesis of AIS possibly by estrogen desensitizing melatonin receptors (*92*). Research is needed to evaluate how the vertebral growth plate response to estrogen may be affected by calmodulin and growth factors including those from platelets.

Calmodulin and estrogen receptors. Calmodulin may affect curve progression by reducing the binding affinity of estrogen receptors in growth plates (*12, 14, 59*).

Estrogen receptors on platelets. The estrogen receptor is present in human megakaryocytes and platelets (*64, 100, 143*). Estrogen appears to inhibit the aggregation of platelets but this inhibition is highly dependent on the PI^{A2} polymorphism of glycoprotein GP IIIa (*13, 110*). Polymorphism in the estrogen receptor gene has been reported in association with both susceptibility to AIS and curve severity (*59, 92, 159*). [*Polymorphisms* are stable DNA sequence variations in which two or more alleles for a given locus each occur in greater than 1% of chromosomes in the population (*1*)].

Melatonin, platelets and nitric oxide. The low plasma melatonin levels associated with progressive idiopathic scoliosis reported by Dubousset *et al* (*33*) and Machida *et al* (*78, 79*) have been suggested to act through the nervous system (*78, 79*). Reduced melatonin antagonism to platelet calmodulin has also been suggested as a mechanism (*10, 33, 71, 72, 78*). The platelet/skeletal hypothesis predicts that *lower* levels of plasma melatonin in AIS lead through reduced scavenging of nitric oxide (*88, 104, 113, 160*) to curve progression sequentially by : a) unscavenged nitric oxide causing more vasodilatation adjacent to vertebral growth plates (*89*) resulting in b) increased platelet activation in susceptible subjects, and consequent c) release of growth factors that abet other mechanisms promoting relative anterior vertebral growth and curve progression. However, most studies have reported no significant change in circulating levels of melatonin in subjects with AIS. A new approach is being developed by Moreau and colleagues (*92-95*).

Melatonin signalling transduction defect and a "P factor". Moreau and colleagues (*92-95*) reported evidence of mutations affecting melatonin signalling transduction within osteoblasts and muscle cells from AIS patients with severe scoliotic deformities. The defect was associated in scoliotic patients with high levels of a circulating protein termed "P factor" *w*hich 'appears essential to initiate scoliosis' (*94*). These new findings raise the prospect of both innovative diagnostic tools and tailored pharmacological approaches to rescue the melatonin signalling defect (*95*). Moreau's concept implies that in some subjects with progressive AIS there is a systemic metabolic defect that leads to a progressive scoliosis deformity through unknown mechanisms (? the nervous system). The concept does not exclude a possible role for activated platelets in abetting the hormone-driven growth of the already mechanically-compromised vertebral endplate physes to promote the relative anterior spinal overgrowth of progressive AIS. Whether or not vertebral growth plate chondrocytes, and/or platelets from AIS subjects have the melatonin signalling defect, or synthesize the "P factor, has not been reported.

5. Collagen-induced platelet activation and bleeding time in AIS

In AIS subjects, tests of platelet aggregation by collagen are not detectably different compared with controls (*39, 140*), but more platelet aggregation was found in patients with smaller curves compared with those with larger curves (*140*). The latter finding is consistent with activated platelet involvement being more in early, and less in later, curve progression. The platelet/skeletal hypothesis predicts that when mechanical micro-insults of deforming vertebral bodies denude the endothelium of the dilated vessels and "lakes" (*89*) (fig 3) and expose collagen and adhesive proteins (*109*), platelet activation will occur. Collagen-induced platelet activation probably involves multiple receptors and mainly glycoprotein GPVI (a major and unique platelet receptor to fibrillar collagen) and the integrin receptor $\alpha_2\beta_1$ on activated platelets (to soluble collagen) (*26, 98, 109, 133, 156*). Calmodulin is associated with GPVI in resting platelets and may be related to the regulation of GPVI-dependent activation (*98*).

In females with idiopathic scoliosis (and paralytic scoliosis) *collagen abnormalities* have been suggested to explain the prolonged bleeding time (*147-149*) but the bleeding time findings for idiopathic scoliosis have not been confirmed (*39, 140*). In normal women the longer bleeding time than that of men may be due to difference in blood vessels or supporting connective tissues (*8*) which may be relevant to girls with progressive AIS.

6. Platelets and female susceptibility to progressive AIS

The platelet/skeletal hypothesis for progressive AIS raises the question : of whether about the time of menarche there are changes in the coagulation mechanisms involving platelets/endothelia that prepare the human female to control her new cyclical uterine bleeding. Platelet counts are higher in women than in men (*9, 17, 75*) but the data for adolescents are limited (*45*). *Cardiovascular disease* shows well-known age and gender differences (*87, 129*) ; the need to study gender differences in molecular and cellular physiology of the heart and blood vessels is now gaining attention with most research focused on the effects of estrogen and estrogen receptors (*87*). Studies of platelet physiology in the menstrual cycle have shown that in women there are cyclical platelet fluctuations not present in men (*38, 62, 106, 143*). We are not aware of any research that has examined platelet function in relation to puberty, gender and the onset of the menses (menarche). The skeletal/platelet hypothesis predicts that in puberty the onset of cyclical platelet fluctuations makes the adolescent female in some way susceptible to progressive AIS curves – possibly involving a molecular predisposition to platelet activation. Some of these putative molecular mechanisms may affect males with progressive AIS.

7. Treatment

The platelet/skeletal hypothesis is firstly, consistent with the trial of melatonin and combinations of other radical antioxidant scavengers (*88*) as preventive measures against the progression of AIS (*33, 78, 79, 113*) and secondly, with tailored pharmacological approaches to rescue a melatonin signalling defect in progressive AIS (*95*). The possibility of exhibiting anti-platelet therapy including targeted therapy (*98, 157*) acting on the platelet release reaction is inappropriate, at present, because of the limited knowledge relating to platelet activation and progressive AIS.

Acknowledgements

We are grateful to Professor S. Heptinstall, Professor of Thrombosis and Haemostasis, Cardiovascular Medicine, University Hospital, Nottingham, UK for discussion and his personal communication. We thank Dr R.W. Kerslake, Consultant Radiologist and Mr B.J.C. Freeman, Consultant Spinal Surgeon both of Queen's Medical Centre, Nottingham, UK for advice on radiological investigations. Mr Lyndon Cochrane prepared the figures. Figure 3 is reprinted with permission from Elsevier in "The relationship between scoliosis and growth" by R.G. Burwell, In : Zorab, P.A. (ed). *Scoliosis and Growth, Proceedings of a Third Symposium,* Churchill Livingstone, Edinburgh and London, 1971, pp 131-150.

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