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# Two types of vascularisation of intramural uterine leiomyomata revealed by corrosion casting and immunohistochemical study

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*The blood supply of myomatous uteri collected upon autopsy was examined. The uterine vascular beds were perfused via afferent vessels with fixative followed by Mercox resin and corroded after polymerisation of the resin. The vascular casts thus obtained were examined using scanning electron microscopy. The vascular system of the uterine fibroids was also examined using immunohistochemical analysis (FVIII, factor VIII-related antigen).*

**Key words: fibroids, corrosion casting, leiomyoma, immunohistochemistry, vasculature**

## INTRODUCTION

Uterine fibroids or leiomyomata are the most common solid tumours in women, affecting at least 50% over 30 years of age [16]. Their high incidence, associated symptoms (pelvic pain, menorrhagia) and side-effects (infertility, loss of pregnancy) present a serious clinical problem. Very little is known of the aetiology of uterine leiomyomas. It is known that there are a number of risk and protective factors for and against fibroid growth related to oestradiol [9, 15] and that there are an increased numbers of sex steroid receptors in fibroids compared with myometrium [2]. Other factors that may be associated with fibroid aetiology include peptide growth factors, such as the insulin-like growth factor (IGF), family and epidermal growth factor (EGF) as well as possible chromosomal and subsequent gene changes [14] within these tumours. The corrosion casting technique combined with scanning electron microscopy (SEM) is the best method currently available for morphological examination of the vascular networks. SEM offers a high resolution and quasi-three-dimensional image. SEM was also used to study the vascular system of intramural leiomyomata [17]. FVIII forms part of the von Willebrand factor complex, plays a part in the coagulation cascade and its expression is reduced in smaller, less mature blood vessels [7]. The vascular staining for this factor is no different from that for other vascular markers, i.e. CD 31, CD34 [3]. The present study has been undertaken to compare the results of studies of the vascular network of human uterine fibroids using SEM and immunohistochemistry.

## MATERIAL AND METHODS

30 uteri were obtained upon autopsy of women aged 25–56 years, deceased due to causes unrelated to disorders of the reproductive system. The study was approved by the Ethics Committee of the Jagiellonian University Medical College. The material was collected 6–24 h after death. Each uterus together with the ovaries and cervical portion of the vagina

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was removed in such a way that relatively long fragments of uterine and ovarian vessels (arteries and veins) were retained.

Immediately after removal, 22 uteri were perfused via the afferent arteries with prewarmed (37°C), heparinised saline (12.5 IU/ml heparin, Polfa, Poland) containing 3% dextrane (70 kDa) and 0.025% lidocaine (Lignocaine, Polfa), until the fluid outflowing via the veins was completely transparent (~5 min). Perfusion was then continued using a solution of 0.66% paraformaldehyde/0.08% glutaraldehyde (Sigma) in 0.1 mol/l cacodylate buffer, pH 7.4 supplemented with 0.2% lidocaine. Finally, the vascular system was injected with 60–80 ml of Mercox CL-2R resin (Vilene Comp. Ltd. Japan) containing 0.0625 mg/ml methyl acrylate polymerisation initiator (Vilene Comp. Ltd.) and the uteri were left in a warm water bath (56°C) for several hours to allow polymerisation and tempering of the resin.

When the polymerisation was completed, the uterine tissues were macerated for 5–6 days by repeated baths in 10% potassium hydroxide at 37°C followed by washing with warm (50–55°C) running tap water. The vascular casts obtained were washed for the next 4–5 days in multiple changes of distilled water under mild vacuum conditions, cleaned in 5% trichloroacetic acid for 1–2 days, washed again in distilled water for 2–3 days and freeze-dried in a lyophiliser (Liovag G2; Aqua Fina, Germany).

The freeze-dried casts were examined macroscopically, gently dissected [18] to expose the vasculature of myomata and stored in an exiccator containing phosphorus pentoxide before microscopic examination. They were then mounted onto copper plates using colloidal silver and "conductive bridges" and coated with gold. The casts were examined using a JEOL SEM 35-CF scanning electron microscope at 20–25 kV.

The vascular beds of the next 10 uteri were perfused with saline and then injected with a solution of acrylic emulsion (Liquitex R, Binney and Smith, USA) [19]. The specimens were collected mainly from large fibroids > 3 cm in diameter. All tissue specimens were fixed in 10% formalin and embedded in paraffin wax. The tissue blocks were sectioned (4 $\mu$m) and section-mounted on aminopropyltriethoxysilane (APES)-coated slides. The endothelial cell marker was factor VIII-related antigen (FVIII). Next the tissue blocks were deparaffinised and hydrogen peroxidase (3%) in methanol was applied to the tissue sections as an endogenous peroxidase block for 10 min. The protein blocking steps included the application of 10% normal rabbit serum prior to the application of the primary antibody (anti Human von Willebrandt factor, Dako) in 20% foetal calf serum. After incubation with primary antibody a biotynyled secondary antibody was applied, followed by horseradish peroxidase-streptavidin conjugate and visualisation by means of chromogen 3-amino 9-ethylchlorcarbazole (AEC, Zymed, USA), which identifies tissue antigens with a red-brown stain. Serial sections were immunostained using the endothelial cell marker. The negative controls used substitution of the primary antibody with the non-immune serum for the polyclonal factor VIII antibody.

## RESULTS

Among 30 uteri prepared for corrosion casting, only 7 yielded casts of acceptable quality. The observation was limited to intramural fibroids, since the vascular casts of subserosal tumours were always considerably damaged.

Two systems of blood supply to intramural uterine leiomomata were observed. Some of the fibroids were centrally almost avascular, although their peripheral vessels were usually arranged in the form of a dense network composed mostly of capillaries or multiple smaller and shorter arteries and veins (Fig. 1), forming a so-called "vascular capsule". The others were supplied by 2 or 3 larger arteries running in company with veins, which penetrated the central areas of the lesion, giving relatively few side branches (Fig. 2). In both patterns the veins were flattened, which was probably a result of compression of the tumour.

Very commonly the vessels penetrated the connective tissue septa, which separated the small tumours from each other (Fig. 3).

In routine histological sections the vessels seem to follow the course of the septa and are not visible in the centre of the tumour. By using immunostaining with a vascular marker (FVIII) large vessels can be observed following the septa and small vessels (capillaries) penetrating the substance of the tumour. Here two types of vascularisation of the uterine leiomyomata can be distinguished, one in the form of dense peripheral "vascular capsule" (Fig. 4) and the other made up of a few vessels with side ramifications, penetrating the lesion (Fig. 5).

## DISCUSSION

The vasculature of fibroids has not been studied in detail and has rarely been studied in a quantitative manner [3, 8]. Earlier structural studies [4, 5, 11, 12] using similar methods of pigment or radio-

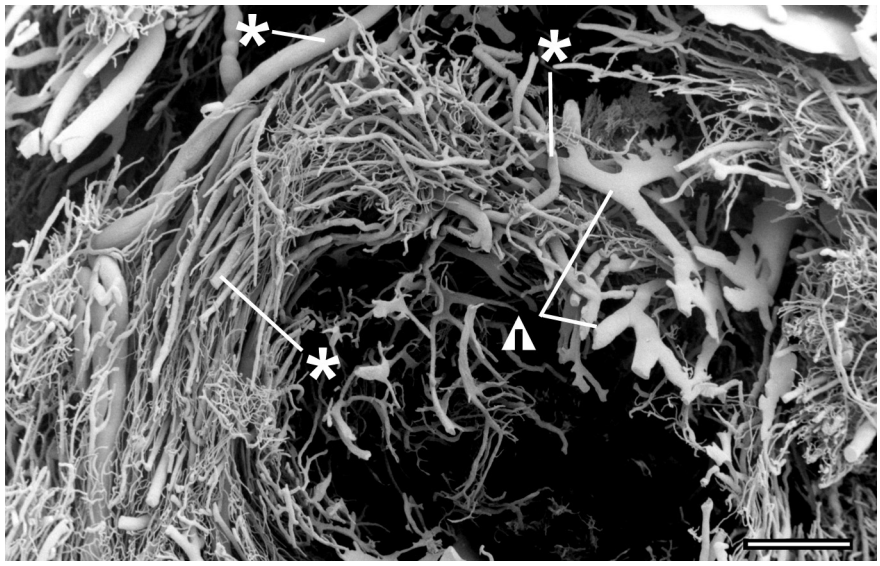

**Figure 1.** Arteries (⁎) and veins (▲) of the "vascular capsule" of small uterine leiomyoma — SEM. Bar = 1000 $\mu$m.

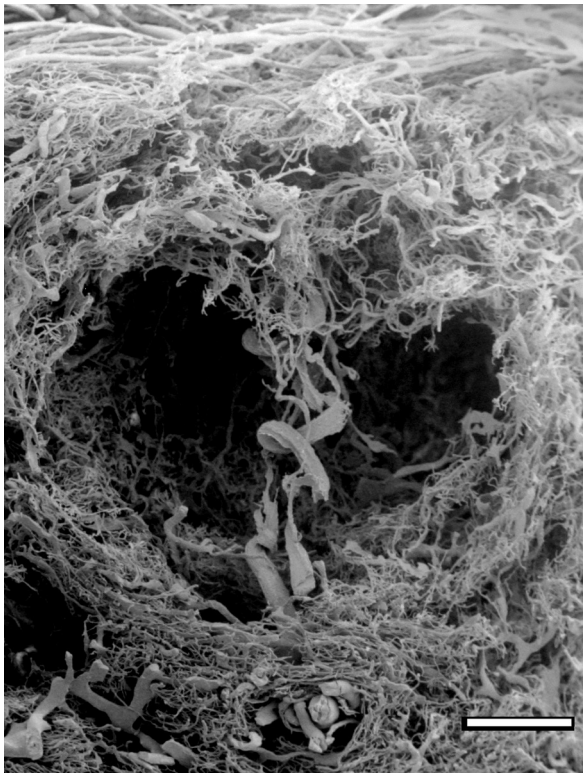

**Figure 2.** Corrosion cast — bundle of vessels (160–230 $\mu$m) penetrating small uterine leiomyoma. SEM. Bar = 1000 $\mu$m.

opaque dye injection demonstrated that the arterial vessels of uterine leiomyoma were typically increased in size, especially in larger tumours, whereas the degree of arterial vascularity was variable. It was also observed that small uterine fibroids were usually, but not always, less vascular than the surrounding myometrium, while larger leiomyomas were usually more vascular than the myometrium. Farrer-Brown et al. [4, 5] also demonstrated that there was no intrinsic vascular pattern in uterine fibroids but rather that the arrangement appeared to represent a localised expansion of the myometrial vasculature with the vessels within these tumours oriented in the direction of the muscle cell bundles. They also showed there were changes in vasculature distant to the tumour, with dilation and congestion of vessels contralateral to the site of the uterine fibroids.

Blood flow studies have been performed in uterine fibroids. The vasculature of uterine leiomyoma was investigated using colour Doppler ultrasound [10, 13]. Their results concurred with the earlier work of Sampson [11, 12], showing that the vascularisation of these tumours was largely dependent on the tumour size, position and the extent of secondary degenerative changes.

It is thought that the growth of a tumour is limited by its blood supply, with tumours dependent upon the ingrowth of capillary sprouts from surrounding tissues for growth beyond a diameter of a few millimetres [1]. The differences in vascular density between myometrium and uterine leiomyomas may represent a difference in angiogenesis and vascular remodelling in these vascular beds. These differences in angiogenesis may be the result of changes in the balance between the factors that promote or inhibit the angiogenesis.

Faulkner [6] in his classic report described the vascular system of the leiomyomata as "a mass of

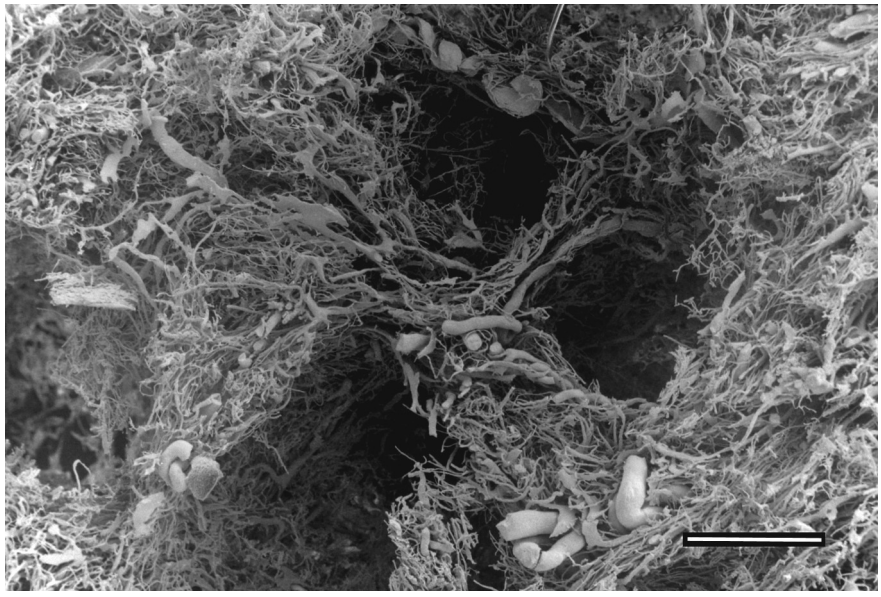

**Figure 3.** Corrosion cast. Vessels of connective tissue septa. SEM. Bar = 1000 $\mu$m.

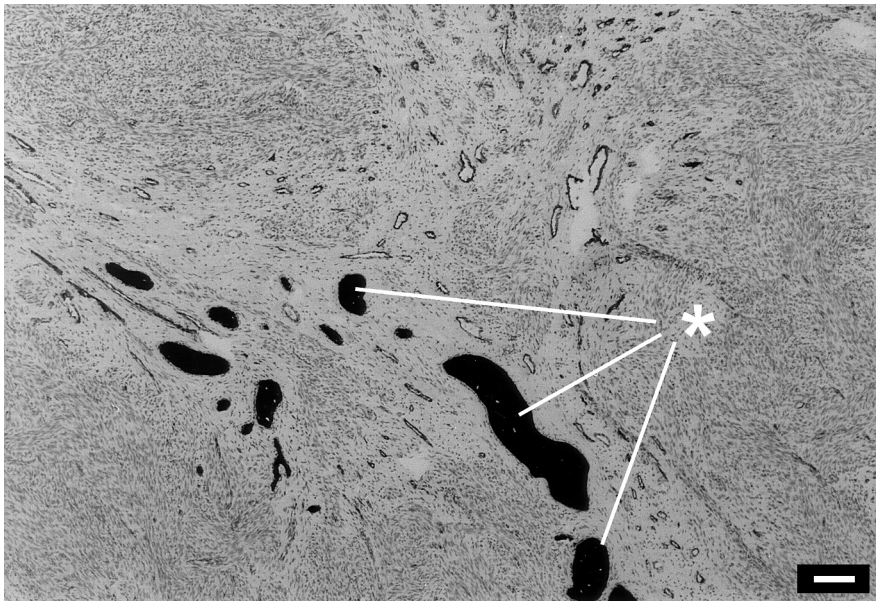

**Figure 4.** Arteries (*) of leiomyoma injected with acrylic emulsion (Liquitex R, Binney and Smith, USA). Numerous empty arteries running through the fibroid septa. Immunostaining for FVIII. Bar = 500 $\mu$m.

proliferating arteries". The technique used by Faulkner did not visualise the capillaries and our studies have shown that arteries are not the dominant vessel type in uterine fibroids. The vascular density of myomata demonstrated by SEM and immunohistochemistry seems to be either lower than or similar to that of unchanged myometrium. In a recent immunohistochemical/morphometric study, Casey et al. [3] reported significantly higher microvascular density in the adjacent myometrium than in small and large leiomyomata. SEM demonstrated the permanent feature of all myomata, which had the form of a "vascular capsule". The immunostaining for FVIII also proved the significantly increased density of peripheral vessels of uterine leiomyomata, thus forming a dense "vascular capsule".

Knowledge of vascular differences may provide information on basic biology, aid understanding of the variability of symptoms reported by women with fibroids, better define the mode of action of some of the current medical managements (gonadotrophin releasing hormone agonists [GnRHa] and RU

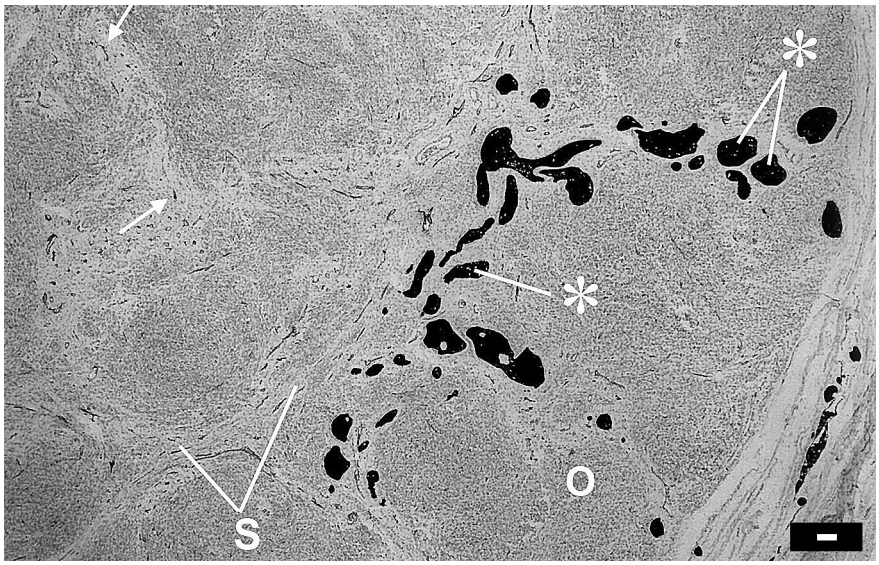


**Figure 5.** Large arteries (*) injected with acrylic emulsion (Liquitex R, Binney and Smith, USA) present in the centre of the myoma. Small empty vessels (↑) — capillaries observable in the centre, O — margin of the myoma. S — septa. Immunohistochemical staining for FVIII. Bar = 500 $\mu$m.

486) and, finally, provide knowledge of the future utility of angiogenesis inhibitors for treatment.

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