RESEARCH

Patterns Microvascular Distribution of the Colonic Wall Studied by Electron Microscopy: Experimental Study in Rats.

S. Sangiorgi [I], L. Boni [II *], G. Dionigi [III], A. Benevento [IV], M. Protasoni [V], A. Manelli [VI], M. Raspanti [VII], Cassinotti E [VIII], F Rovera [IX]

ABSTRACT

Background: The corrosion casting technique, known as the best method to study the three dimensional architecture of blood vessels in many organs, applied to the lower gastro intestinal (GI) tract was used to visualize the morphological patterns of small blood vessels supplying the colonic wall at scanning electron microscopy (SEM).

Methods: 15 Wistar male rats weighing 300 g were deeply anaesthetised by an intraperitoneal injection of ketamine. An acrylic low viscosity resin was injected through the abdominal aorta before the origin of celiac trunk once it reached the superior and inferior mesenteric arteries; it filled marginal arteries, submucosal plexus, the perforant capillaries. The specimens were then observed in scanning electron microscope.

Results: The vascular architecture of the colon that can be divided as followed: the major vessels, vessels supplying both longitudinal and circular muscular layers, the microvessels of submucosal plexus, and the mucosal vessels.

Each layer of the colonic wall present its own vascular pattern with some difference regarding the mesenteric and antimesenteric site.

Interpretation: Corrosion casting technique has demonstrated to be a useful method for the detailed comprehension of the vascular architecture of an organ as colon.

Each layer of the colic wall present different and probably functional pattern that can be variable in the mesenteric or antimesenteric side.

INTRODUCTION

The vascular anatomy of the large bowel have been widely studied by means of macro and microscopic techniques and the pathways of the major vessels as well as the lymphatic channel is well known.

On the other hand there are only few studies on the distribution of morphological patterns and topographical disposition of the microvessels inside the colonic wall in rats [1,2]. Such information might result in some importance (i.e. kinetics of cancer growth and metastases related to the site of origin) especially with regard to the differences among the sites of the colonic wall.

Corrosion casting technique (CCT) is known as the best technique for the study of the three dimensional architecture of blood vessels in many organs both in pathological and in physiological conditions [3, 4, 5, 6].

The aim of this experimental study is to describe the patterns of small vessels that supplies blood to the colonic wall in all its layers, using CCT and subsequent scanning electron microscope (SEM) examination. Differences among the different colonic wall layers (muscular, submucosal and mucosal) as well colonic sides (mesenteric and antimesenteric) have been recorded.

MATERIAL AND METHODS

All these procedures are in accordance with the Guide for Care and Use of Animal Experimentation of the University of Insubria in Varese.

15 Wistar male rats weighing 300 g were deeply anaesthetised by an intraperitoneal injection of ketamine (75mg/kg).

Xifo-pubic midline laparotomy was carried out and the abdominal aorta exposed under a dissecting stereomicroscope (Leica WILD M3C).

A 21G cannule was then inserted in the aorta near the iliac bifurcation and driven until the uprising of inferior mesenteric artery. The cannule was then fixed by proximal and distal ligatures on the vessel.

A vascular clip was also applied to the thoracic aorta to prevent the diffusion of the resin in the upper part of the animal body and to assure a way-out in case of a sudden increase of injective pressure. The caval vein was then sewed to allow blood outflow.

The inferior and superior mesenteric arteries were then identified and the lower intestinal tract was perfused with 30 ml of heparinized saline solution to prevent blood clotting followed by an injection of 20 ml of saline to complete the wash out of the remaining blood from all the vascular bed.

Slow injection of 10 ml of Karnovsky's solution (0.25% glutaraldehyde and 0.25% paraformaldehyde in 0.1M Na-cacodylate buffer at pH = 7.2) was carried out to fix the vascular bed in order to prevent the leakage of the resin and to reduce the modifications occurring to the endothelial cells during the injection of the medium.

30 ml of acrylic resin (MERCOX™ – SPI supplies, USA) mixed with 25% of methyl methacrilate monomer was added to 0.2 ml of catalyzer (benzoyl peroxide) and the mixture obtained was manually injected through the cannula until reflux from the venous vessels became evident and the viscosity of the resin appeared gradually increased. The cannula was then removed from aorta and a ligation was carried out in order to prevent any reflux of the resin.

After partial polymerization of the resin, all the entire bowel was explanted and immediatly immersed in a warm water bath (60°C) in order to complete the polymerizing process for about one hour.

Afterwards, the colon was identified, isolated and finally put in a 15% KOH solution to digest all the tissues around vessels. The digesting solution was changed daily after washing each time all the specimens in distilled water.

One week later the resulted casts were then immersed in a solution of formic acid to clean them and at last dissected under a stereomicroscope.

The obtained specimens were then treated for SEM observations by dehydration in graded alcohol, Critical Point dried in an Emitech K850 CPD apparatus, mounted on aluminium stubs on adhesive film and coated with 10 nm of gold in an Emitech K250 sputter-coater .

Some specimens, due to their dimensions, needed some metallic bridges, to maintain a good electric conduction all over the stub.

The specimens were then observed in Scanning electron microscope Philips XL-30 FEG at 10 kV.

RESULTS

In all 15 rats analyzed we found the same vascular architecture of the colon that can be divided as followed: the major vessels derived from mesenteric arteries and surrounding the colon itself giving rise to all the vascular support of this organ; the vessels supplying both longitudinal and circular muscular layers, the microvessels of submucosal plexus, and the mucosal vessels.

Each of these regions have their own morphological characteristics that might correspond not only to a specific histological architecture but also to a precise physiological duty, as described below.

Major vessels

The vascular supply of the colon comes from superior and inferior mesenteric arteries, that, after giving origin to arciform arteries end into medium sized arteries that surround from both sides the external surface of colonic wall (Fig.1).

From these vessels some perforant arterioles arise, directed towards the underlying layers and representing their vascular afferent support.

Muscular layers

The vessels supplying the muscular layers of the colonic wall are mostly represented by capillaries arranged along the two axes of the organ and following the natural disposition of muscular fibres.

They form two dense layers with a medium intervascular distance of 7 μm: the external longitudinal one and the internal circular one.

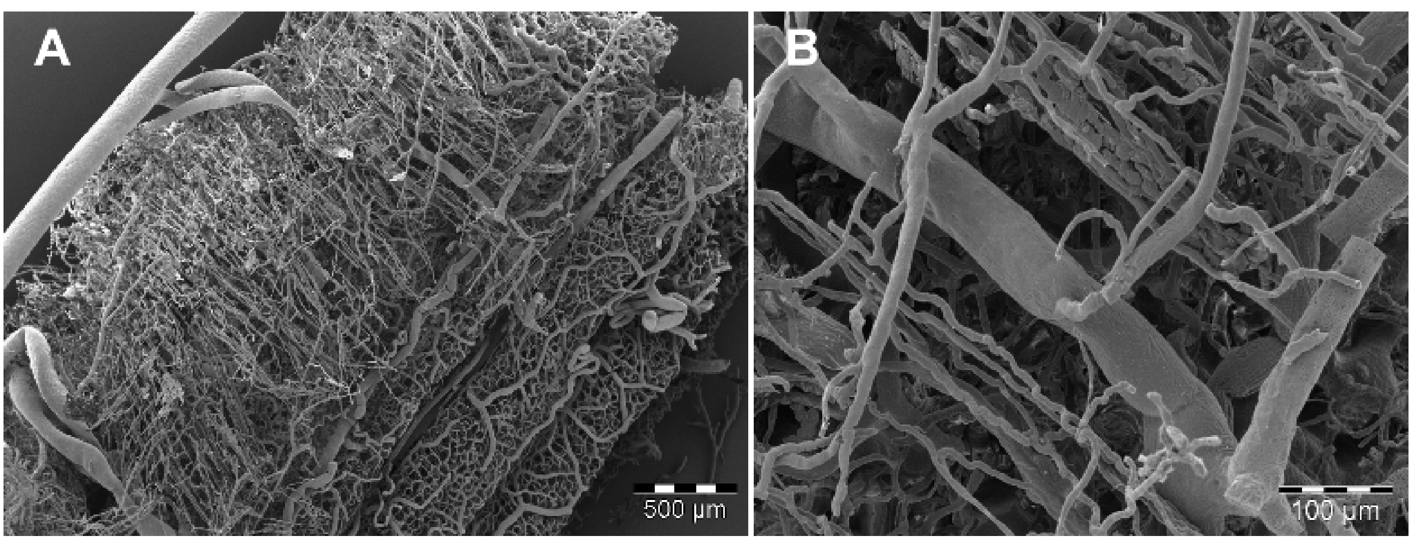

Figure 1. (A): Low magnification of colonic microvasculature: note the incomplete filling of muscular layer in contrast with the visible complete filling of submucosal and mucosal capillaries.

Figure. 2. (B): High magnification of muscular vessels arisen from “T shaped vessels” arranged in a more superficial sheet of longitudinal capillaries and a deeper transversal one.

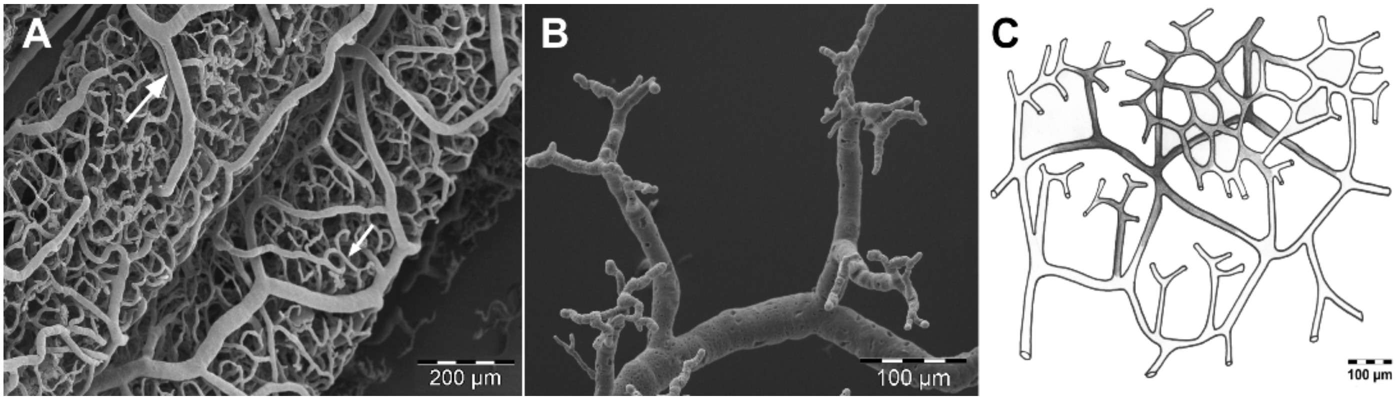

Figure 3: Submucosal layer: wide polygonal net of medium sized (big arrow) and capillaries (thin arrow) seen from the external side of the colon. From this layer arise some perforant vessels directed towards the mucosal layer and giving origin to its vascular architecture (B) as represented in the scheme (C).

In the external muscular layer the capillaries are arranged longitudinally and parallel to the major axis of the organ: the vessels arise as "T" shaped vessels directly from the arciform arteries (Fig.2).

In the internal muscular layers the capillaries have a circular disposition and form a square angle with those belonging to the upper layers: in this region the capillaries are very thin and measure about 5 µm in diameter. We found only few connections between these capillaries that run parallel one to another along all their pathway.

Sometimes these capillaries loose their stretched shape and become spiral. Moreover we could observe some blind ending vascular tips and many patterns resembling constrictions: ring-like ones and extended along the longer axis of the vessel.

Significant differences have been found between the mesenteric and anti-mesenteric site of the colon: the first present a dense capillary net that does not appear in the anti-mesenteric site (yellow arrow).

Submucosal layer

Regarding the submucosal layer, we observe a regular tridimensional net of medium sized vessels, organized to form polygonal rings, measuring about 400 µm in diameter, with sides that vary from 100 to 200 µm (Fig. 3 A, B, C).

From each vertex of the polygon one perforant vessel bears perpendicularly to this layer and penetrates throughout the entire wall till the internal mucosal surface which gives

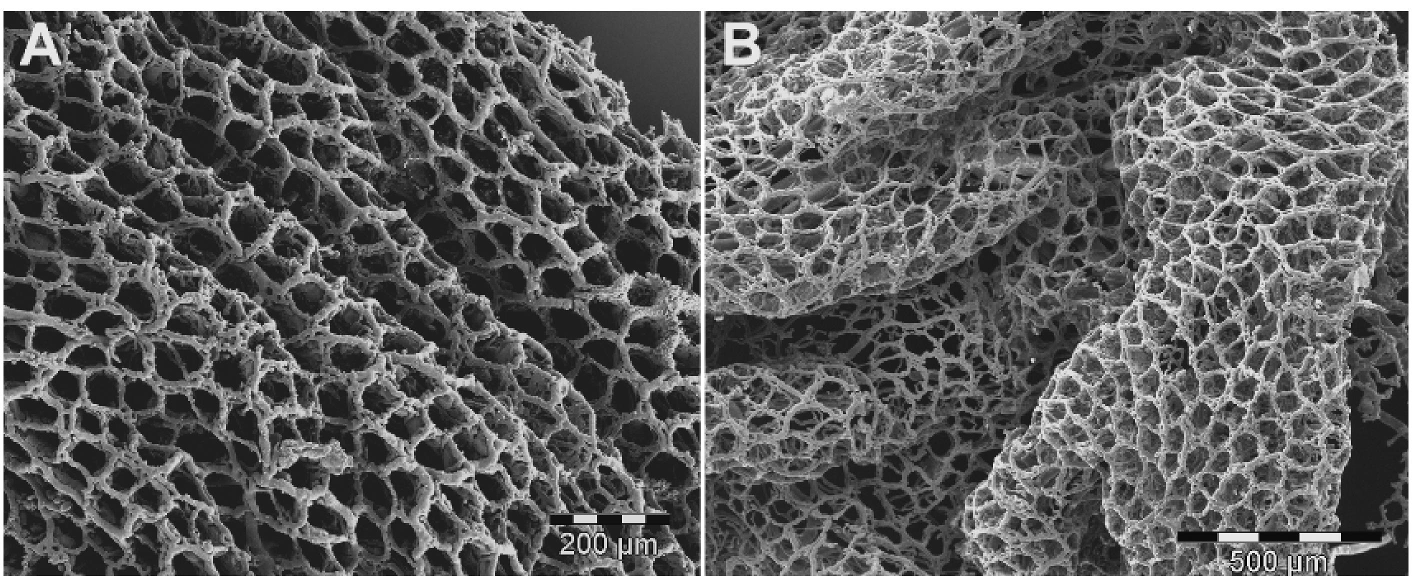

Figure 4: Mucosal layer: low magnification of honeycomb-like structure of colonic mucosa (A): note the regular dispositions of polygonal rings that follow the natural shape of superficial plicae (B).

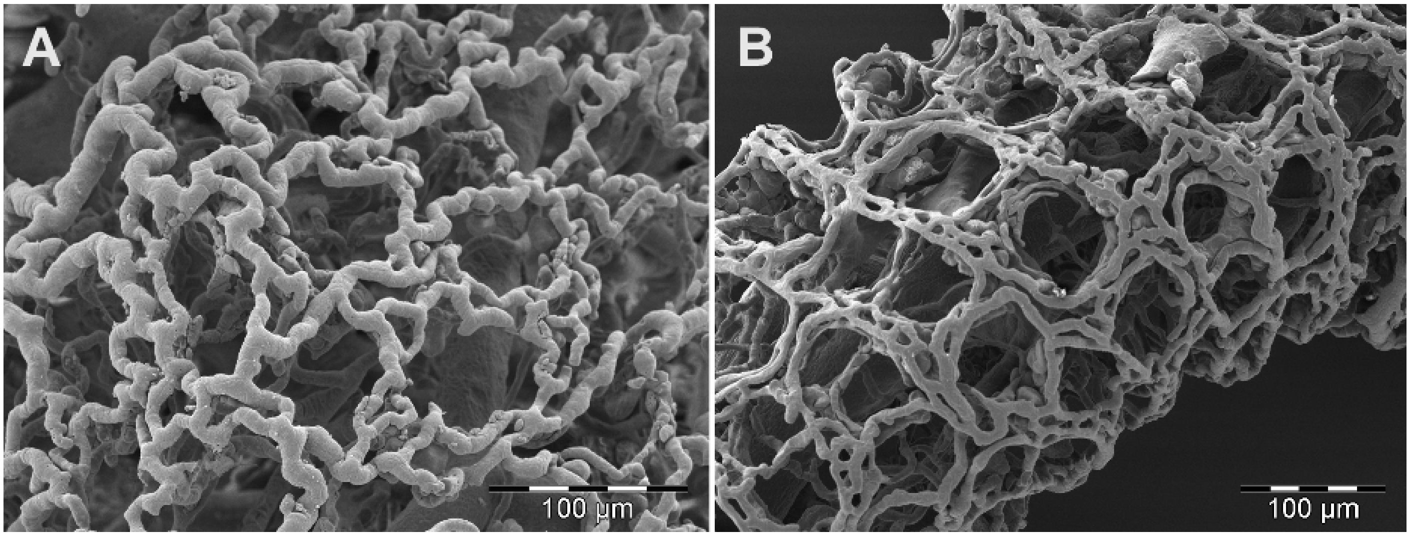

Figure 5: High magnification of the vascular structure of colonic mucosa in relaxed (A) and stretched (B) conditions. Note in the first case the extremely tortuosity of the sides in mucosal rings that becomes stretched and straightened during distension.

origin to the superficial net of capillaries.

Before reaching the surface, these vessels split into three or four branches supplying each hexagonal ring of its vertex. These perforant vessels are 100 µm long and measure about 10 µm in size (Fig.3 B/C).

In this layer we can observe on the cast single ring-like grooves that reproduce the reduction in the vascular calibre made by an isolated sphincteric system.

Sometimes it is also possible to see longitudinal quite deep grooves left by a more elongated sphincteric systems, lying all over the external surface of the vessel and causing a constant reduction in diameter along the major axis of the vessel itself.

The constrictions become more frequent in the deep major vessels supplying the submucosal layer that penetrates the muscular sheets reaching the junctions with the interstitial matter.

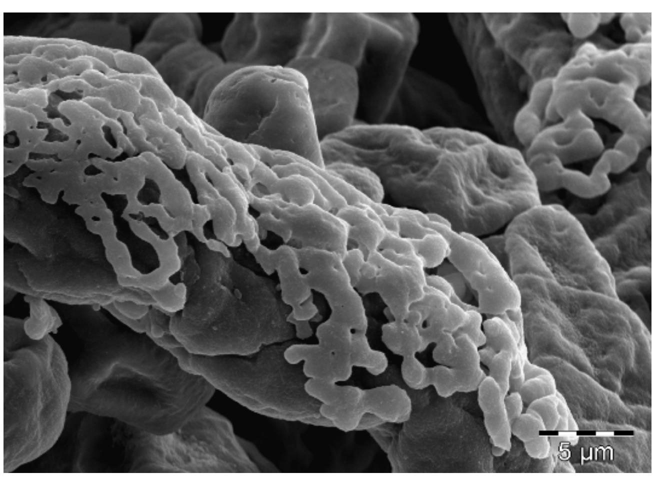

Figure 6: Evidence of extravasation of the resin in lymphatic pathways: in this case the lymphatics seems to lie on enveloping a capillary.

Mucosal layer

The vascular architecture of colonic mucosa follows the normal disposition of its plicae normally and it does not change for size and morphology (Fig. 4 A/B).

It is composed by a wide net of capillaries arranged in hexagonal rings linked one to another along each of their sides that generate a honeycomb-like microvascular net. Each of these vascular units measures about 70 µm in diameter while the side is 25 µm long (Fig. 4A).

Each mucosal hexagonal ring give rise to a single capillary that usually form a tortuous plexus along the wall of the mucous gland reaching its bottom (Fig. 4B).

All the capillaries constituting the superficial mucosal rings may have two different conformations: they can be tortuous or more stretched (Fig.5 A/B).

Sometimes it is also possible to observe the lymphatic pathways cast with the resin.

They mostly appear in the form of irregular spheroidal agglomerates enveloping capillaries (Fig.6).

DISCUSSION

For a long time, the ink-gelatine injection and the observation of specimens at light microscope was the only method to study the microcirculation in many organs. Nevertheless using this technique it is only possible to obtain low resolution images lacking any information about the three dimensional disposition of the vessels that can only be hypothesised by serial precise sections.

On the other hand corrosion casting technique coupled with scanning electron microscope investigation can easily obtain high-resolution three dimensional images of blood capillaries after chemical digestion of the surrounding tissues [4, 5, 6].

The intrinsic viscosity of the injecting medium (50 centipoise) allows a detailed view of the smallest capillaries constituting the vascular architecture of many organs in order to study their morphological conformation, intervascular distances, morphological patterns and variants [7].

One of the most investigated site is the mucosal plexuses of viscera, because of their implications in many pathophysiological conditions.

However, to date, there are no studies that describe by a topographic point of view the variants occurring in vascular disposition of each layer constituting the colonic wall and, in particular, that compare its vascular architecture enhancing the differences existing not only among these layers but also between the mesenteric or antimesenteric pole.

In our study, at low magnification, the first appearance of vascular structure of the colon it was the extremely density of capillaries mostly sited in the muscular layers.

This fact was accompanied by the evident arrest of the resin in correspondence to the midline between mesenteric and anti-mesenteric pole leading to a significantly different vascular pattern of the two side at the level of the muscular layer.

In a previous experimental and clinical study [8,9] we demonstrated that colonic cancer, growth on one of the two side, have a different pattern of diffusion and findings of the present study might partially explain clinical and experimental results.

Even if the resin stopped, the submucosal and mucosal capillaries of both sides were completely filled. This fact can well demonstrate how independent could be the vascular structure of muscular layers and mucosal-submucosal one.

This empiric observation make us to imagine that a preferential blood flow direction exist towards mucosal and submucosal layer in comparison to the muscular one.

On the other hand it should be considered that the resistance of the vascular circuit in the muscular layer is higher than in the mucosal one because of the thinner diameters of muscular capillaries in comparison to the mucosal ones.

Analysing the mucosal layer that faces the colonic cavity, we could distinguish the regular honeycomb like superficial net, that it could be related to some physiological condition such as morphological modification during the peristaltic activity since the mucosal side is the first to be submitted to the pressure created during the peristaltic motion. This pressure causes a gradual modification in the disposition of vessels starting from the extension of the side of the mucosal hexagon till the whole elongation, along the axis of the acting force, of the honeycomb-like structure. Thanks to such morphology and to the elasticity of the tissues around, the mucosal capillaries can be easily elongated during the physiological extension of the organ, and return tortuous during relaxation.

Furthermore, the vascular architecture of the mucosa has an intrinsic morphological structure and elasticity that assures a peculiar resistance to stretching forces, allowing to distribute the increasing pressure just along this layer.

Once the pressure increases, other vascular structure plays an important role in absorbing the stress. The vascular support of mucous glands, for example, can be longitudinally stretched during the distension. Moreover, the angle existing between perforant capillaries and submucosal net reduces itself more and more, till zero.

At high magnification, we could distinguish some ultrastructural details, in these vessels: on capillaries, we usually find the nuclear impression that, on bigger vessels can be useful to distinguish an artery from a vein (this depends on the disposition of nuclear impression, longitudinally oriented in arteries and randomly placed on veins).

Sometimes, we can find the faithful reproduction of corrugated endothelial cell's membrane, due to the stress produced by the injecting medium.

It is possible also to come across some artefacts, that is important to know, not to misunderstand important informations: plastic ring structures present around the vessels, sign of extravasation as for the lymphatic pathways' casting, present if the pressure of injection exceeds.

Blind endings can be found as a sign of blood clots or bubble air formed during the mixing of catalyser.

In conclusion, this technique has demonstrated to be a useful method for the detailed comprehension of the vascular architecture of an organ as colon, frequently implied in pathological disorders, such as neoplastic and ischemic ones, giving us a detailed vision of the topographic disposition of its vascular patterns.

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I. First Author: S. Sangiorgi, M.D.
Department of Neurosurgical Unit – University of Insubria

II. L. Boni, M.D.
Department of Surgical Sciences – University of Insubria

III. G. Dionigi, M.D.
Department of Surgical Sciences – University of Insubria

IV. A. Benevento, M.D.
Department of Surgical Sciences – University of Insubria

V. M. Protasoni, M.D.
Department of Human Morphology – University of Insubria

VI. A. Manelli, M.D.
Department of Human Morphology – University of Insubria

VII. M. Raspanti, M.D.
Department of Human Morphology – University of Insubria

VIII. Cassinotti, M.D.
Department of Surgical Sciences – University of Insubria

IX. F Rovera, M.D.
Department of Surgical Sciences – University of Insubria

Corresponding Author:
Lougi Boni, M.D.
Department of Surgical Sciences, University of Insubria
Viale Borri 57
21100 Varese – ITALY
E-mail: luigi.boni@uninsubria.it

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