

**ORIGINAL STUDY**

**STRUCTURAL MICROANATOMICAL CHANGES IN BRONCHIAL  
ASTHMA**

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**ABSTRACT**

*Airway remodeling in asthma is a complex process involving structural changes in almost all tissues of the airway walls. Along with respiratory tract inflammation this is one of the main pathological features of asthma. The aim of the study was to evaluate semi-quantitative microvascular characteristics, as part of the remodeling process in bronchial asthma. Study group consisted of 8 patients diagnosed with asthma, deceased. Lung fragments were collected in the necropsy. There was a striking change in the orientation of microvessels, from a position that was parallel to the surface of the epithelium to a position perpendicular to the surface of the epithelium. The density of the microvessels amplified progressively in the same time with the increase of the asthma condition (GINO classification).*

**KEYWORDS:** *asthma, remodeling, semi-quantitative characteristics.*

**1. Introduction**

Airway remodeling in asthma is a complex process involving structural changes in almost all tissues of the airway walls. Along with respiratory tract inflammation this is one of the main pathological features of asthma. The onset of this process remains unknown, and this turning point could be seen as a potential therapeutic target in asthma. Remodeling occurs in both adults and children, the relationship between airway inflammation and airway remodeling are still controversial. Currently, there are no therapeutic interventions to reverse airway remodeling.

Histological changes in the respiratory tract are: proliferation and differentiation of epithelial goblet cells, sub-epithelial fibrosis of airway smooth muscle (ASM), increase in angiogenesis, protein matrix deposit, hyperplasia and hypertrophy of the glands, nerve proliferation. This paper refers only to the pulmonary angiogenesis in asthma.

**2. Material and Methods**

The aim of the study was to evaluate semi-quantitative microvascular characteristics, as part of the remodeling process in bronchial asthma.

Study group consisted of 8 patients diagnosed

with asthma, deceased. Lung fragments were collected in the necropsy.

Some tissue fragments were processed by standard procedures for immunohistochemical examination using CD34 as a microvascular marker.

Semi-quantitative analysis was performed by PRODIGIT 5.2. by analyzing vascular density.

Protocol for the preparation of the tissue fragments:

- 24 hours in buffered formaldehyde;
- processed in paraffin;
- pieces of 4 microns serial sections;
- paraffin removal for those sections;
- section staining;
- endogenous and non-specific peroxides blocking;
- incubation overnight at 40 ° C with anti-CD34, with a dilution ratio of 1:100;
- immune reaction was amplified using appropriate secondary antibody and Streptavidin- biotinylated - peroxidase (HRP) complex;
- the sections got under 3.3 diaminobenzidine tetrahydrochloride chromogen;
- Hematoxylin Mayer staining;
- control for negative reaction in each antibody.

Semi-quantitative assessment of microvascular density was performed by 40x image and after establishing the field by 200x image.

The following classification was performed depending on semi-quantitative assessment of microvascular density (MVD):

- a slight increase in blood vessels number or a small hypervascularity (MVD <25)
- average hypervascularity (MVD ranging between 25 and 35)
- strong growth in the number of blood vessels or severe hypervascularity (MVD > 35)

### 3. Results

After assessing microvessels of angiogenesis

from lamina propria there were established the following hypervascularity types:

Type 1. Densification of microvessels in the surface area (under the immediate underlying basic membrane, proper for asthma type I and II);

Type 2. Constant and uniform distribution of the microvessels in the entire lamina propria with a perpendicular position on the epithelium area (instead of a parallel position), which are characteristics in asthma III and IV.

Application of semi-quantitative analysis resulted into:

- slight increase in the number of blood vessels in all cases diagnosed with intermittent asthma; (MVD=164,12 microvessels/mm<sup>2</sup>),
- the average number of vessels in the lamina propria was similar in patients with mild persistent asthma compared with those diagnosed with intermittent asthma; (MVD= 193.4 microvessels /mm<sup>2</sup>),
- moderate increase in vessel density in all cases of moderate persistent asthma (MVD=211,2 microvessels /mm<sup>2</sup>);
- significant increase in vessel density in all cases diagnosed with severe persistent asthma (MVD=318.8 microvessels /mm<sup>2</sup>).

### 4. Discussions

In this study we used the GNA classification for assessing asthma severity (table I ) [1].

Angiogenesis can be defined as the formation of new vessels by sprouting from pre-existing vessels. Angiogenesis is a component of the chronic inflammatory response that occurs in the bronchial wall, which seems to be initiated by FGF, TNF- $\alpha$  and VEGF. Evaluation of mucosa microvascularization opens new prospects for advanced therapies with beneficial effects for the quality of life in patients with asthma [2,3]. A better understanding of the hypervascularity mechanism in asthma brings

prospects for its inhibition with a response in the thickness of the bronchial wall and reduction of swelling. From this perspective, it was demonstrated that corticosteroids had a beneficial role in reducing angiogenesis. Inhibition of neo-angiogenesis is mediated by inhibition of the growth factors, mainly EGF [4]. Moreover, methylprednisolone inhibit angiogenesis and other factors, such as IL-8, IL-18, MMP 9. However, it can certainly support the beneficial role of inhaled corticosteroids in reducing the number of blood vessels in the bronchial wall [5-7].

**Table I.** Classification of GNAA in bronchial asthma

	Daytime symptoms	Overnight symptoms	PEF or VEMS PEF Variation
Stage I Intermittent	S< 1/week PEF normal asymptomatic between crisis	≤ 2 /month	≥ 80% <20%
Stage II Slightly persistent	> 1/week but > 1/day Crisis can affect everyday activity	> 2 /month	≥ 80% 20-30%
Stage III Moderate persistent	Daily Crisis affect the cavity	> 1/week	60-80% >30%
Stage IV Severe persistent	Permanent Limited physical activity	Frequent	≤60% >30%

Angiogenesis must be taken as a complex multi-stage process involving a large number of growth factors, cytokines, chemokines, enzymes and other factors (table II):

VEGF-R2 is a major mediator of the mitogenic effects, angiogenic and permeability. Angiopoietin-1

stabilizes the new vessel as angiopoietin-2 in the presence of VEGF, acts as an antagonist of Ang-1 making the blood vessels fragile and increasing the germination of vessels. Endostatin is a potent inhibitor for endogenous angiogenesis. Metalloproteinases (MMPs) are a large family of zinc and calcium-dependent peptidases which are capable of degrading most of the tissue components of the extracellular matrix [8-10].

**Table II.** Inflammatory factors and Growth factors

Transmitters	Histamines, Tryptase, Chymase, Heparin, Carboxypeptidase A, MMP-2, MMP-9
Cytokine	IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-13, TNF $\alpha$
Chemokine	RANTES, Eotaxin, MCP-1, MCP-3, MCP-4, IL-8
Growth factors	VEGF, bFGF, TGF $\beta$ , GM-CSF, PDGF, PAF
Lipids-derived compounds	PGD <sub>2</sub> , LTB <sub>4</sub> , LTC <sub>4</sub> , LTD <sub>4</sub> , LTE <sub>4</sub>

This function is an essential requirement to allow cell migration in tissue, extending existing vessels, and sprouting of new vessels (table III).

Mast cells are known as one of the most important sources of proangiogenic factors and may secrete many mediators involved in angiogenesis, such as VEGF, bFGF, TGF $\beta$ , MMP, histamine, chemokines (IL-8 in particular), cytokines, and proteases [11,12].

Electron microscopy studies have shown that in patients with asthma, the lower respiratory tract show fenestrated capillaries, increasing the vascular permeability and allowing the plasma extravasation, which contributes to the formation of the luminal mucus stoppers.

Plasma leakage can also lead to edema and thickening of the bronchial wall, reducing the airway

lumen, which will minimize the flow of air and may contribute to airway hyper-responsiveness [13,14].

The increase in number of microvessels facilitates the development of edema followed by thickening in the bronchial wall and concomitant reduction of the distensibility [15,16].

**Table III.** *Inflammatory factors involved in angiogenesis*

Angiogenesis	Vasodilatation	Permeability
VEGF	VEGF	VEGF
FGF	Histamine	Histamine
TGF $\beta$	Heparins	Adenosine
HGF	Tryptase	Bradykinin
HIF	NO	Ang-1, Ang-2
Ang-1	TGF $\alpha$ , TGF $\beta$	SP
Histamine	FGF	CGRP
PGD <sub>2</sub>	EGF	LTB <sub>4</sub> , LTC <sub>4</sub> , LTD <sub>4</sub>
PGI <sub>2</sub>	IL-4	PAF
LTC <sub>2</sub>	TNF $\alpha$	ET-1
PAF	LTC <sub>4</sub>	TNF $\alpha$
SP	PGD <sub>2</sub>	ECP
VIP		
IL-8, IL-13		
TNF $\alpha$		
NKA		
Angiogenin		
MMPs		
IGF-1		
Chymase		

The high density of capillaries is probably related to a higher metabolic rate in airway

epithelium, which is very active in the secretor process. In fact, the oxygen consumption of the airway epithelium is comparable to that of the liver and heart. In normal airways, vascular homeostasis is the result of complex interactions between numerous pro-and anti-angiogenic. The bronchial flow can be affected by alveolar pressure and lung volume [17].

The thickening of the airway wall can be caused by microvascular hyperplasia, the enlargement of the smooth bronchial muscle mass or by hypertrophy of mucus secreting glands [18].

Each of these changes can be induced by the inflammatory cells that infiltrated into the airway wall, in both severe and non-severe forms of asthma.

Cytokines, chemokines and growth factors from inflammatory cells and structural cells contribute to the pulmonary remodeling process [19].

Mast cells and lymphocytes may be important originators and controllers of these processes, while activated eosinophils are key cells in the reactor. Their presence in large numbers is associated with increased reactivity in the airway.

Physiological consequences in remodeling process are hyper-responsiveness in airways by fibrosis increase and hyper secretion of mucus or mucus-secreting cell hyperplasia.

The increased level of the blood flow in bronchial asthma was demonstrated by measuring the exhaled dimethyl ether, which showed an average increase in blood flow to 24-77%. This increased level resulted from angiogenesis and growth in vascular density [20].

#### 4. Conclusions

There was a striking change in the orientation of microvessels, from a position that was parallel to the surface of the epithelium to a position perpendicular to the surface of the epithelium.

The amplification of the microvascularisation

was initiated in the superficial lamina propria spreading through the entire surface of the bronchial mucosa.

The density of the microvessels amplified progressively in the same time with the increase of the asthma condition (GINO classification).

Morphological results showed a prominent development of the vascular bed in severe persistent asthma compared with mild asthma.

Although our methodology was different, this study is consistent with other studies and the results are consistent with previous reports.

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