

## Special Stains To Histologically Study Left Atrial Appendage (Laa) - Orcein & Ptah

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### Abstract:

#### Background

The structure of the heart undergoes constant adaptation to physiologic changes in the organism. The left atrium possess a venous component, a vestibule and an appendage and shares the septum. LAA is a major endocrine organ and is the main producer of ANP (atrial natriuretic peptide) in the human heart. Atrial cardiocytes of the mammalian heart contain granules which are morphologically similar to those in peptide secreting endocrine cells. The cardiocytes of LAA contain the greatest density of ANF granules found in Left Atrium and it is therefore of interest to carry out further studies on these granules.<sup>3</sup>

#### AIMS

Looking to the great clinical significance that is given to the morphology of LAA & its predilection as a site for thrombus formation, we've conducted a detailed study histologically of LAA. To investigate LAA and study the arrangement of elastic bundles of LAA histologically (with a special stain-Orcein) and also study of the arrangement of muscle bundles of LAA histologically (with a special stain-PTAH), these findings were co-related with that described in the literature.

#### Materials & Methods

The materials for the present study were collected from the Department of Anatomy & Forensic medicine of Sri Aurobindo Medical College and Post Graduate Institute, Indore. Randomly five hearts were selected for histological purposes of Left Atrial Appendage (LAA) which included the following:

Slides of LAA from different sites (apex, inferior margin and atrio-auricular junction) were prepared by routine Histological Techniques and stained. By Haematoxylin & eosin and Orcein (special stain) & PTAH (special stain).

#### CONCLUSION

This study of its histological features is an attempt to establish the cause of various pathologies related to arrhythmias.

**Keywords:** : A.N.P., L.A.A., Orcein, PTAH

## **Introduction**

It is axiomatic that an understanding of the complex architecture of the atrial musculature should improve understanding of its activation and contraction. Describing Harvey as the 'patron saint' of anatomists, Arthur Keith, in his Harveian Lecture given in 1918, referred to the architecture of the musculature of the heart as a 'well-worn theme' of the 17th century. In his earlier treatise written in 1907 on the jugular pulse, Keith had opined that each atrium and ventricle contained two sets of muscular fibres—circular and longitudinal. He suggested that the circular fibres in the right atrium were for compressing the chamber and expelling the blood, while the longitudinal fibres were antagonists of those to be found in the right ventricle.<sup>9</sup>

The left atrium possess a venous component, a vestibule and an appendage and shares the septum. The Left Atrial Appendage (LAA) is characteristically a small finger like extension in human hearts and has crenations over all lobes that are potential sites for disposition of thrombus.<sup>1</sup>

For decades anatomy text books tended to focus on the ventricles because of their important role as pumping chambers. The two atria have long been looked at as collecting chambers, hence attracted less interest for studying them.

Actually, the LAA is a remnant of the original embryonic left atrium formed during the third week of gestation. The LAA lies within the pericardium in close contact with the free wall of the left ventricle. It is therefore likely that blood flow, in and out of the LAA, depends to a significant degree on a properly functioning left ventricle.<sup>5</sup>

The trabecular LAA is a remnant of the original embryonic left atrium that develops during the third week of gestation. The main

smooth-walled left atrial cavity develops later and is formed from an outgrowth of the pulmonary veins. The appendages passively fill during ventricular systole and then passively empty during ventricular diastole.<sup>2</sup>

Atrial cardiocytes of the mammalian heart contain granules which are morphologically similar to those in peptide secreting endocrine cells. These secretory granules were discovered by Kisch (1956). These granules are known as Atrial Natriuretic Factor (ANF).<sup>6</sup>

LAA is a major endocrine organ and is the main producer of ANP (atrial natriuretic peptide) in the human heart. The ANP concentration is 40 times higher in the LAA walls than in the rest of the atrial free wall and in the ventricles. A study of patients having undergone the maze procedure and associated LAA removal found a significantly lower ANP secretion and a commensurate increase in salt and water retention. Whether this could eventually lead to hypertension is not known.<sup>5</sup>

So, analysis of ANF has shown that LAA contain about 30% of all cardiac ANF. The cardiocytes of LAA contain the greatest density of ANF granules found in Left Atrium and it is therefore of interest to carry out further studies on these granules.<sup>3</sup>

### **AIMS and OBJECTIVES**

Looking to the great clinical significance that is given to the morphology of LAA & its predilection as a site for thrombus formation, we've conducted a detailed study histologically of LAA.

- To investigate LAA and study the arrangement of elastic fibres in LAA histologically (with a special stain-Orcein) and these findings were co-related with that described in the literature.

- To investigate LAA and study the arrangement of muscle bundles of LAA histologically (with a special stain-PTAH) and these findings were co-related with that described in the literature.
- The specimens were photographed and photomicrography of appropriate histological sections was carried out.
- These findings were correlated with those cited in the literature. An attempt was made to draw a correlation of these findings with the functions of LAA.
- These in turn help in understanding on its clinical significance and would be of help manage these patients.

#### **MATERIALS & METHODS**

The materials for the present study were collected from the Department of Anatomy & Forensic medicine of Sri Aurobindo Medical College and Post Graduate Institute, Indore. Randomly five hearts were selected for histological purposes of Left Atrial Appendage (LAA) which included the following:

For histology: slides of LAA from different sites (apex, inferior margin and atrio-auricular junction) were prepared by routine Histological Techniques and stained.

#### **II. HISTOLOGY**

Small pieces (2.0cm X 0.5cm) of LAA were taken from different sites (apex, inferior margin and atrio-auricular junction). The tissues were fixed in 10% formalin, and were processed for routine histological procedures - like dehydration, clearing and after wax embedding, blocks were prepared. Sections 5-6micron thick were cut and stained with the following stains:<sup>7,8</sup>

- i) H & E: Method for staining are-
1. Sections were dewaxed with xylene and the sections were taken to water (with descending grades of alcohol).
2. Stain in haematoxylin, in a jar for 5-10minutes.
3. Wash well in running tap water for 2-3minutes.
4. Remove excess stain by decolorizing (differentiating) in 0.5-1% hydrochloric acid in 70% alcohol for few seconds.
5. Wash in alkaline running tap water for 5minutes.
6. Stain in 1% aqueous eosin for 1minutes.
7. Wash surplus stain in water.
8. Dehydrate in alcohol, clear in xylene.
9. Mount in D.P.X. or Canada balsam.
- ii) P.T.A.H. (Phospho-tungstic-acid-haematoxylin): Method for staining are-
1. Take sections to water.
2. Treat with iodine and sodium thiosulphate.
3. Place in 0.25% aqueous potassium permanganate for 5 minutes.
4. Wash in water for 2 minutes.
5. Place in 5% oxalic acid for 10 minutes.

6. Wash in water for 5 minutes and rinse in distill water.
7. Stain in P.T.A.H. for 4-6 hours.
8. Dehydrate in 95% alcohol.
9. Dehydrate in absolute alcohol, clear in xylene and mount in D.P.X
- iii) Orcein: Method for staining are-
1. Dewax the sections.
2. Bring sections to 70% alcohol.
3. Stain in the orcein solution for 30minutes -2hour.
4. Wash thoroughly in 70% alcohol.
5. Differentiate in 1% acid alcohol for destaining of collagen fibers.
6. Wash thoroughly in tap water.
7. Counterstain nuclei with methylene blue.
8. Dehydrate in alcohol.
9. Clear in xylene.
10. Mount in D.P.X.<sup>7</sup>

#### **OBSERVATIONS**

The Left Atrial Appendage (LAA) is a very unique structure within the pericardial cavity, although small in size and having a variegated appearance, it is notorious for arrhythmias and thrombo-embolic phenomenon. This study was undertaken with the objective that certain features of histological anatomy may help in elucidating certain special features of LAA which can be correlated with its tendency for malfunctioning.

#### **HISTOLOGY**

Histological features of the three regions (atrio-auricular junction, inferior margin and apex) studied showed almost identical features with only very slight variations that have been included in the observations described below. Due to the same reason, only selected photomicrographs have been included. Following are the microscopic findings:

#### **Epicardium**

- The outer layer of the heart is termed epicardium. There was a thin layer of epicardium where deep to it greater amount of adipose tissue was present. These features were seen in all the three sites studied namely atrio-auricular junction, inferior margin and apex.
- Deep to the lining epithelium were layers of fibrous tissue intermixed with elastic fibers. (fig1.). Similar findings were seen in sections taken from apex and inferior margin.
- Where the wall of the left auricle was very thin sub-epithelial tissue was continuous with sub-endocardial tissue with only thin connective tissue being present between them. Epicardium showed a thin layer of elastic fibers in sections taken from inferior margin (fig.1).
- On a deeper plane variable amount of adipose tissue was present. Similar findings were seen in sections taken from apex and inferior

margin. In the sub-epicardial region many large blood vessels and nerves were present (fig.).

- Lining cells of epicardium in (Atrio-Auricular Junction) AAJ showed the presence of flattened cells which at places were cuboidal to columnar, whereas at some places, in sections of apex and inferior margin lining of cuboidal cells was seen.

#### b) Myocardium

- Myocardium of AAJ in the auricular wall showed variable thickness. Myocardium was very vascular (fig.). Very prominent transverse striations were seen in longitudinal sections of muscle fibers (fig.1a). Similar findings were seen in sections taken from apex and inferior margin.
- Size of the fibers was variable. Fibers were oriented in different directions: where the atrial wall was thin the fibers were mostly circularly arranged. At places, longitudinal fibers were sandwiched between transversely oriented fibers.
- There was presence of large number of blood vessels of various sizes and types seen between the muscle fibers (fig.). They were present in sections of all the three sites studied.

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- There was presence of large number of blood vessels of various sizes and types seen between the muscle fibers. They were present in sections of all the three sites studied.
- Musculi pectinati were seen as projections in the auricular cavity being lined by endocardium. In musculi pectinati there was presence of longitudinal and transverse fibers. Similar findings were observed in sections taken from apex and inferior margin.
- Presence of granules, which were of deep purple color, were observed in peri-nuclear area in some of the muscle fibers. Similar findings were there in sections taken from apex and inferior margin.

#### c) Endocardium

- The endocardium is the innermost layer that lines the chambers of the heart. Endocardium of AAJ was not uniform; it was wavy in character where it was thin. This was also, seen in sections taken from apex and inferior margin. Elastic fibers, which were non-cellular and eosinophilic, were seen (fig.3). Well-defined zonation could be observed in sub-endothelial connective tissue (fig.3), which showed wavy elastic fibers also. Elastic fibers were present along with variable amount of connective tissue. There was stratification of elastic lamella (fig.3). These features were also seen in sections taken from apex and inferior margin.

- The atrial wall endocardium was smooth with flattened endothelial cells. At places sub-endocardial space with loose connective tissue was observed (fig.2).
- In addition, in the deeper part of the endocardium at places rounded empty looking cells were present. These were also found in sections taken from apex and inferior margin. In one of the sections of inferior margin muscular artery was seen embedded in sub-endocardial region (fig.).

The atrial wall endocardium was smooth with flattened endothelial cells. Whereas, auricular wall showed projections. Where amount of loose connective tissue increased in the deeper part of endocardium, there were a number of undulations seen in the endocardial lining. At places sub-endocardial space with loose connective tissue was observed. There was absence of sub-endocardial space in some regions, so that the endocardium was almost adherent to myocardium.

In sections taken from apex, at places, musculi pectinati came close to each other thereby separating part of the auricular cavity.

**Fig. 1(a):** Photomicrograph of section of Atrio-auricular junction showing transverse striations in longitudinal muscle fibers. (PTAH; 40x)

[Image Placeholder: Description - Photomicrograph showing transverse striations in longitudinal muscle fibers under PTAH stain at 40x magnification.]

#### SECTION SHOWING VESSELS WITH H&E STAIN IN SUB-EPICARDIAL REGION

[Image Placeholder: Description - Section showing vessels with H&E stain in sub-epicardial region.]

**Fig.1:** Photomicrograph of section of Inferior margin showing elastic band (blue arrow) in the epicardium. Thin epicardium (blue arrow) and thick epicardium (black arrow) are seen. (Orcein;10x)

[Image Placeholder: Description - Photomicrograph with elastic band in epicardium under Orcein stain at 10x, arrows indicating thin and thick epicardium.]

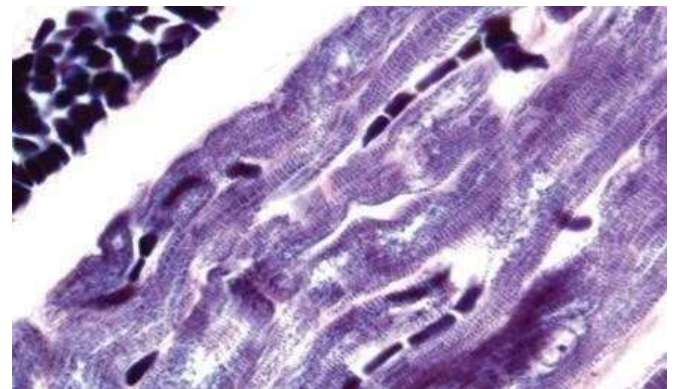
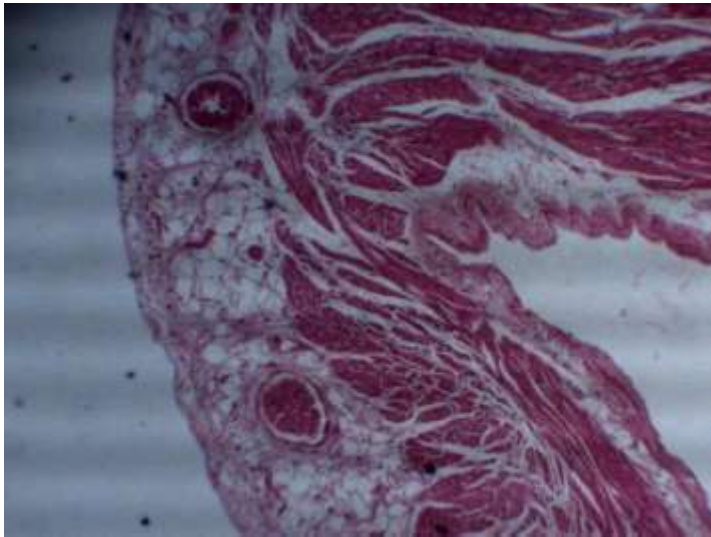


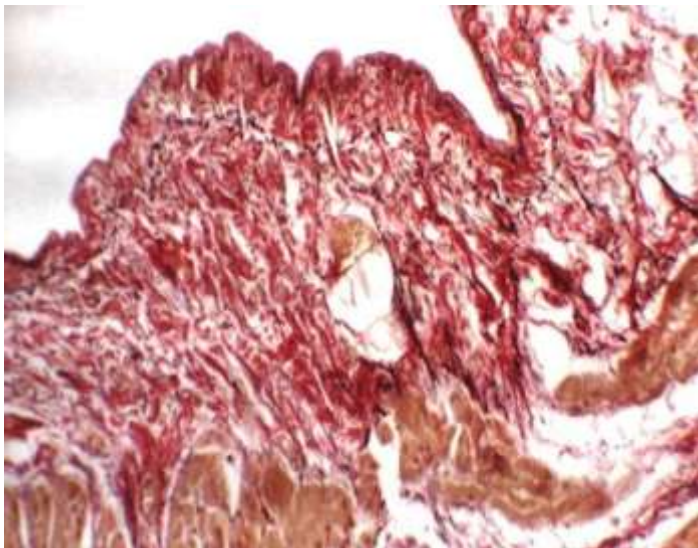
Fig. 1(a): Photomicrograph of section of Atrio-auricular junction showing transverse striations in longitudinal muscle fibers. (PTAH; 40x)



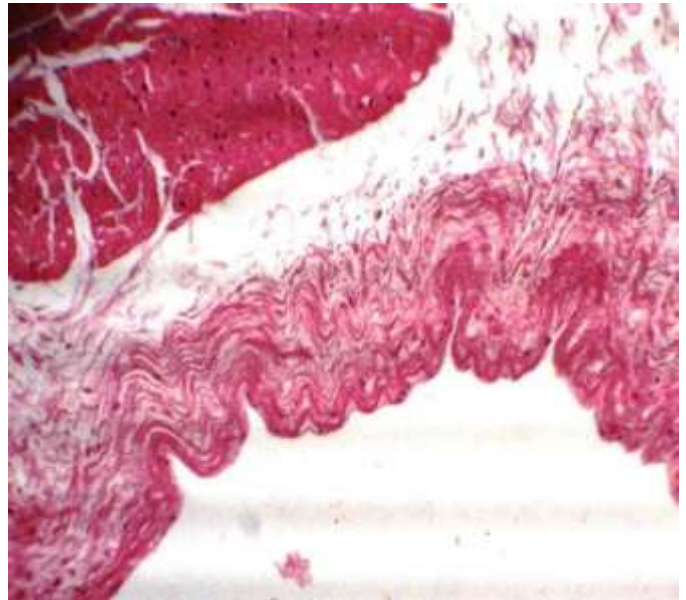
**SECTION SHOWING VESSELS WITH H&E STAIN IN SUB-EPICARDIAL REGION**

**Fig.2:** Photomicrograph of section of Inferior margin showing thick endocardium with elastic fibers (black arrows) and loose connective tissue in sub-endocardial space(blue arrow). (H&E;10x)

[Image Placeholder: Description - Photomicrograph showing thick endocardium with elastic fibers and loose connective tissue under H&E stain at 10x.]



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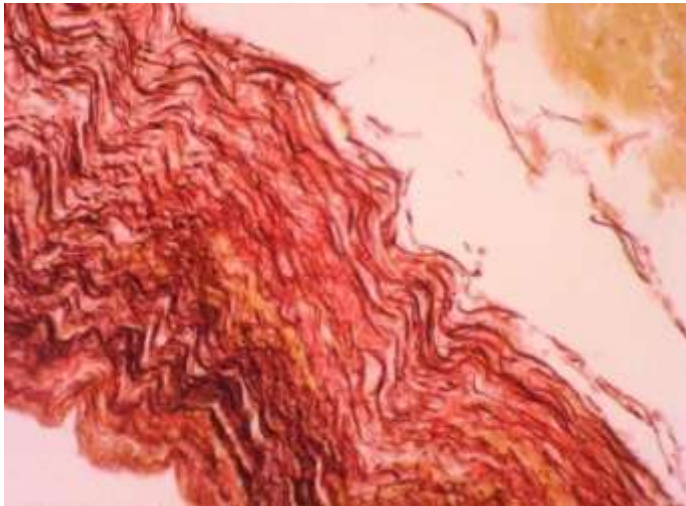


• Fig.2: Photomicrograph of section of Inferior margin showing thick endocardium with elastic fibers (black arrows) and loose connective tissue in sub-endocardial space(blue arrow). (H&E;10x)

**Fig. 3:** Photomicrograph of section of Atrio-auricular junction showing thick elastic fibers in the endocardium. (Orcein; a-10x, b-40x)

[Image Placeholder: Description - Photomicrograph showing thick elastic fibers in endocardium under Orcein stain at 10x and 40x.]





**Fig. 3: Photomicrograph of section of Atrio-auricular junction showing thick elastic fibers in the endocardium. (Orcein; a-10x, b-40x)**

#### **Summary and conclusion**

This study is an attempt to establish various dimensions of LAA and study its histological features so that they can be correlated to establish the cause of various pathologies related to arrhythmias. The shapes of shapes and depths that can become the potential sources for thrombus formation leading to embolic phenomenon. The histological features of all the three regions of LAA studied confirm to the classical description of all these layers of the atrial wall, with a few exceptions: i) sub-endocardial tissue showed stratification along with a number of elastic lamellae; This study of its histological features is an attempt to establish the cause of various pathologies related to arrhythmias. The histological features of all the three regions of LAA studied confirm to the classical description of all these layers of the atrial wall, with a few exceptions: i) some special category of cells were seen within the connective tissue of the endocardium; ii) sub-endocardial tissue showed stratification along with a number of

elastic lamellae; iii) thick cardiomyocytes showed the presence of fine granules in the peri-nuclear zone; iv) at places the epicardium was lined by cuboidal to columnar cells.

The present findings will be of great help in the interpretation of Trans-Esophageal-Echocardiography (TEE) and the understanding of the thrombo-embolic phenomenon

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