

UNIVERSITÀ DEGLI STUDI DI PAVIA

FACOLTÀ DI INGEGNERIA

DIPARTIMENTO DI INGEGNERIA INDUSTRIALE E
DELL'INFORMAZIONE

Bachelor degree in Bioengineering

PROCESSING OF DATA FROM TENSILE TESTS
ON SAMPLES OF ASCENDING AORTA
ANEURYSM

Elaborazione di dati di quantità meccaniche da test
di trazione
su campioni di aneurisma dell'aorta ascendente

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Grazie a..

Chapter 1

Intro

intro

1.1 Introduction

Currently cardiovascular diseases (CVD) represent the major cause of death in Western countries. Among CVD, aneurysm of the ascending aorta is one of the most difficult pathology to diagnose; prone to dissection or even tearing of the wall of the aorta is one of the most deadly aortic disease mostly because it is almost asymptomatic. Aneurysm is in fact usually silent unless an imaging study has been performed; thus, true incidence is hard to estimate. Lethal thoracic aortic dissections/ruptures are also often misdiagnosed. Nowadays, many sources of error are encountered regularly in the clinical practice of aortic care as, for example, an inherent level of resolution of current imaging technologies and limitations of specific imaging modalities [1]. Critical to decision-making in aortic surgery is an understanding of when complications (dissection/rupture) occur in the natural history of unoperated thoracic aortic aneurysms. To know when these complications occur would permit rational decision-making regarding elective, preemptive surgical intervention. However, at present, there is no full understanding of the AsAA evolution and there is a long way to go before getting an established and Most heart surgeons have rudimental means of decision-making whether to surgically operate on AsAA based on the aneurysm diameter, the threshold of which is indicated by the ESC (the so-called “maximum diameter criterion”) which is 5.5 cm. Often in medicine this threshold is a floating value which can decrease or increase depending on the clinical condition of patients causing uncertainty of therapies.

This work only focusses on the mechanical properties of AsAA and statistically analyse experimental data obtained from uniaxial tensile tests on samples excised from 46 patients.

The final goal of this research is to add data and informations to the literature currently available in order to further help in developing a rational specific medical treatment of aneurysm based on proven reasons. Investigating the mechanical properties (thickness of the aneurismal aortic wall, strain at break, stress at break and maximum Young’s module) of dilated aortic tissue may help in the prediction of aneurysm complications, especially when the obtained experimental data are combined with clinical, genetic, and histological information. This work tries to understand relations between the recorded mechanical quantities from tensile tests and two factors: age and gender of the patient.

Statistical analysis has been made in order to understand the data; rational methods (ANOVA, MANOVA etc.) have been used in order to give a standardized method of analysis of aneurysmal samples; mathematical proof results have been achieved but with all the considerations that have to be made in a statistical-study environment. This work starts from the clinical and medical point of view of the problem, continues with the engineering data testing and concludes with statistical studies and results, trying to give decisive support in the understanding of the ascending aorta aneurysm behaviour and mechanical properties.

An active collaboration between the IRCSS Policlinico San Matteo of Pavia and an engineering unit of the Department of Civil Engineering and Architecture of the University of Pavia made this work possible.

Chapter 2

Ascending aorta

aorta

The aorta is the main vessel which supplies the entire body with fresh oxygenated blood pumped out of the heart; the ascending aorta (AAo) is one of the first portions of this main vessel [2] (highlighted in fig. 2.1).

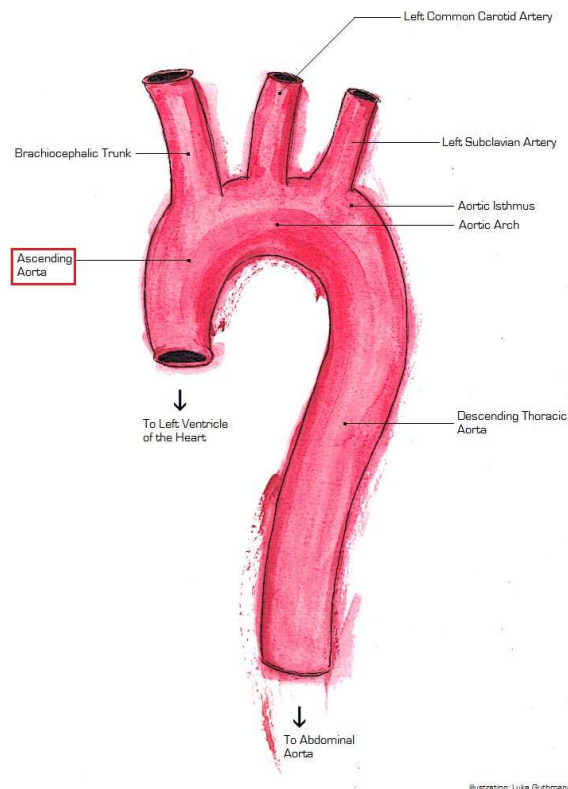


Figure 2.1: Aortic arch

2.1

http://upload.wikimedia.org/wikipedia/commons/a/a0/Aorta_Anatomy.jpg

2.1 Anatomy

- Position and shape

Ascending Aorta (AAo) [3] originate from the upper part of the left ventricle (height of the lower border of the third costal cartilage under the sternum left half) and passes upward

obliquely then forward and to the right directed towards the heart's axis where it ends at the second right costal cartilage (fig. 2.2). With a curved shape and circa five centimeters long, the ascending aorta is located six centimeters behind the posterior surface of the sternum.

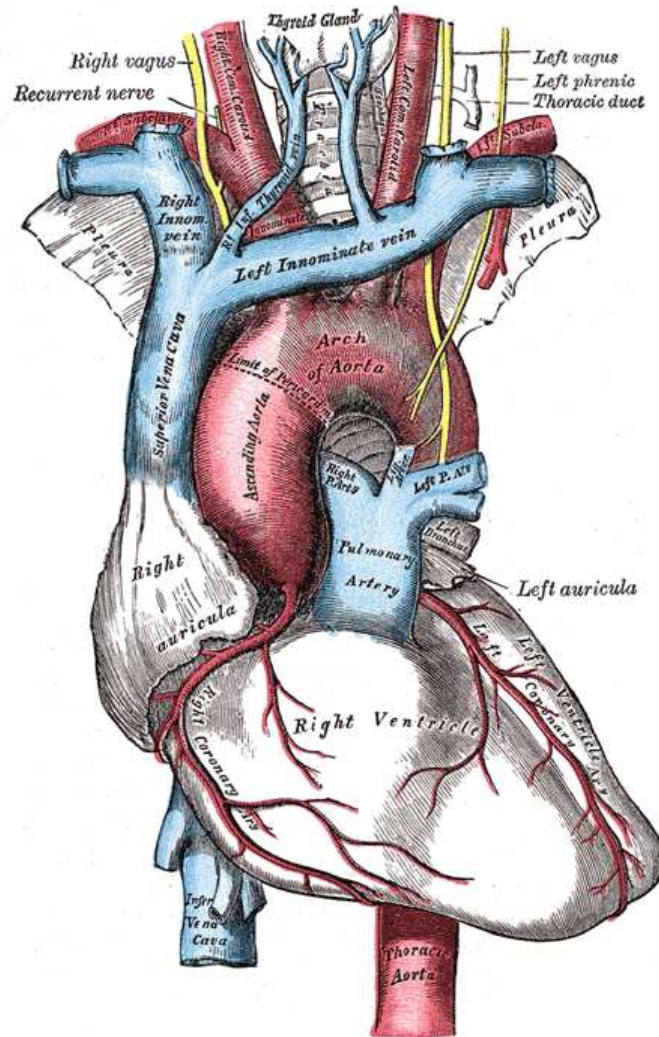


Figure 2.2: Position of the aorta
<http://www.bartleby.com/107/142.html>

2.2

- **Segments**

The aorta begins with an aortic root in the aortic annulus and ends at the sinotubular junction. This particular shape (see fig. 2.3) is due to the aortic sinuses, three small dilatations opposite the aortic valve. The sino-tubular junction is the endpoint of the aortic sinuses. The remainder of the aorta is a tubular structure.

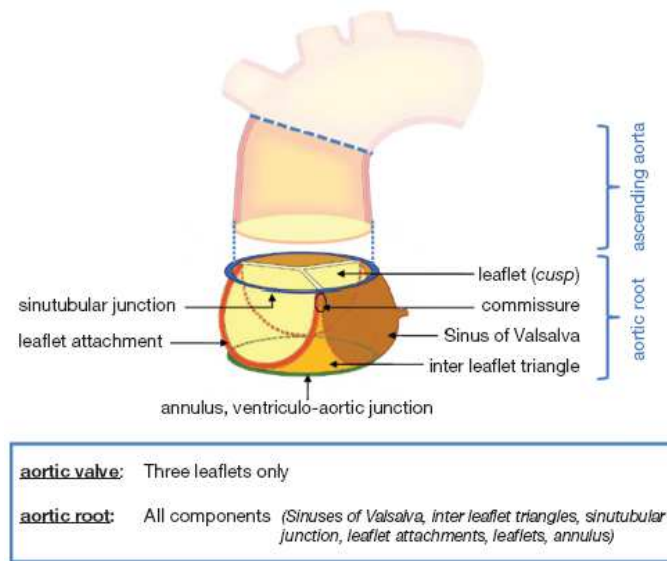


Figure 2.3: Aorta initial tract

<http://www.annalscts.com/article/view/1400/2025>

2.3

- **Branches**

From the ascending aorta only the coronary arteries (fig. ^{2.4}_{2.4}) emerge and these vessels have the vital function of carrying blood to the heart.

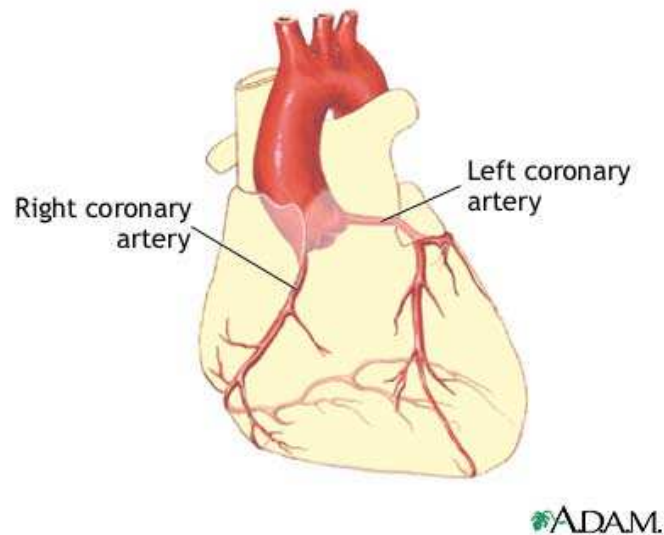


Figure 2.4: Coronaries

<http://www.nlm.nih.gov/medlineplus/coronaryarterybypassurgery.html>

2.4

- **Relations**

The ascending aorta is enveloped by a serous pericardium shared with the pulmonary artery. The latter, plus the right auricula, cover it at the start (fig. ^{2.5}_{2.5}). Under the sternum, the pericardium, the right pleura, margin of the right lung, areolar tissue and part of the thymus separate the aorta from the bone. The ascending aorta lies on the left of the right atrium,

on the superior vena cava and the pulmonary artery.

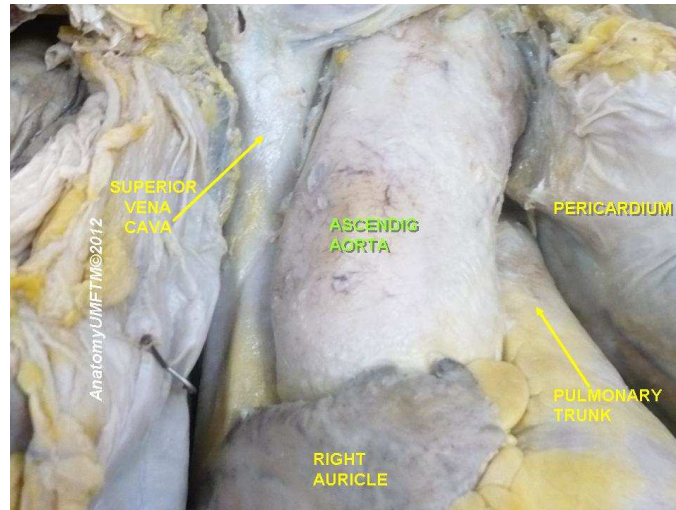


Figure 2.5: Position of the ascending aorta

<http://upload.wikimedia.org/wikipedia/commons/5/5b/Slide2www.JPG>

2.5

2.2 Histology

Endothelium, smooth muscle, elastic elements and connective tissue are the main components that, arranged in concentric layers (see fig. 2.6), form the vascular walls. These layers have undergone characteristic segmental differentiations [4] for each type of vessel and they are (in-out order):

1. Tunica intima

This inner layer comprises the endothelium, the adjacent basement membrane which both, in order, lay on top of the subendothelial connective tissue and the internal elastic lamina.

2. Tunica media

The middle layer is composed of smooth muscle cells, elastic lamellae and includes the external elastic lamina and collagen fibres.

3. Tunica adventitia

The external layer contains connective tissue in which cells like macrophages, fibroblasts and mast cells are located together with nerves and vessels that supply the vascular wall; the latter are called vasa vasorum and they supply the outer half of the vessels.

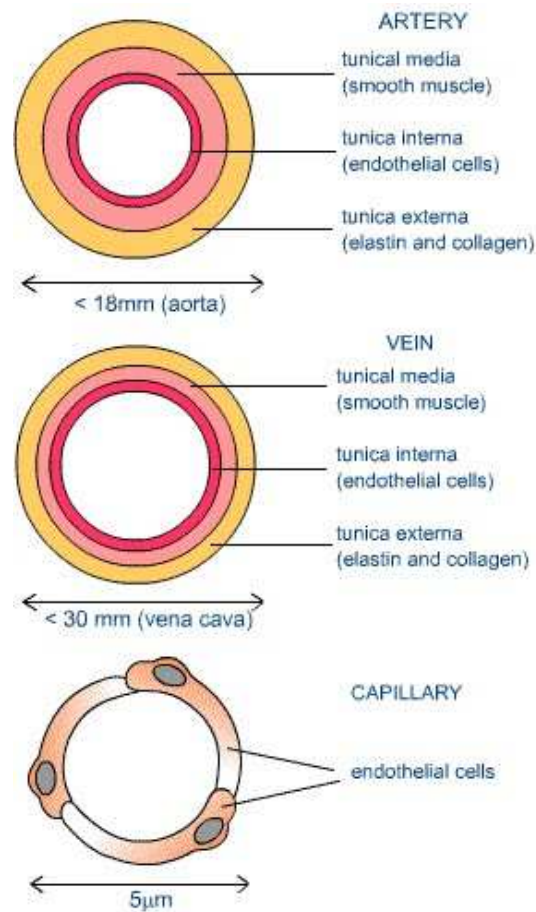


Figure 2.6: Histology of different vessels

2.6

<https://internationalgcsebiology.wikispaces.com/Transport+in+humans>

2.2.1 Aortic Histology

Arterial layers follow the above-mentioned composition (fig. 2.8) but they slightly differ (fig. 2.7).

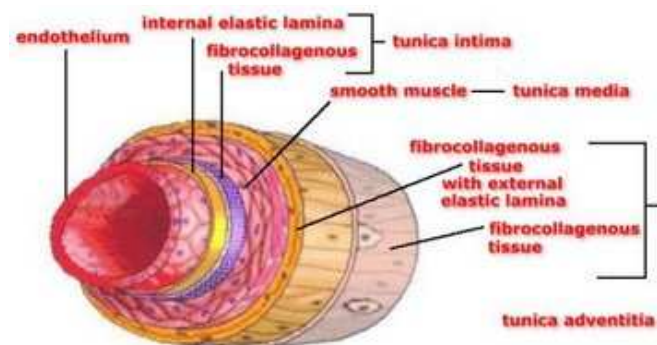


Figure 2.7: Artery layers

2.7

<http://www.perryphysicaltherapy.com/arterial.html>

Ascending aorta, like all macro-vasculature, can be seen with the naked eye and appears as an isolated anatomical entity (differentiating itself from microvessels that appear as parts of the tissue

they supply, in both structural and functional ways). It belongs to the class of elastic arteries which is indeed primarily represented by the large vessels emerging from the heart ventricles such as the descending aorta and the pulmonary artery.

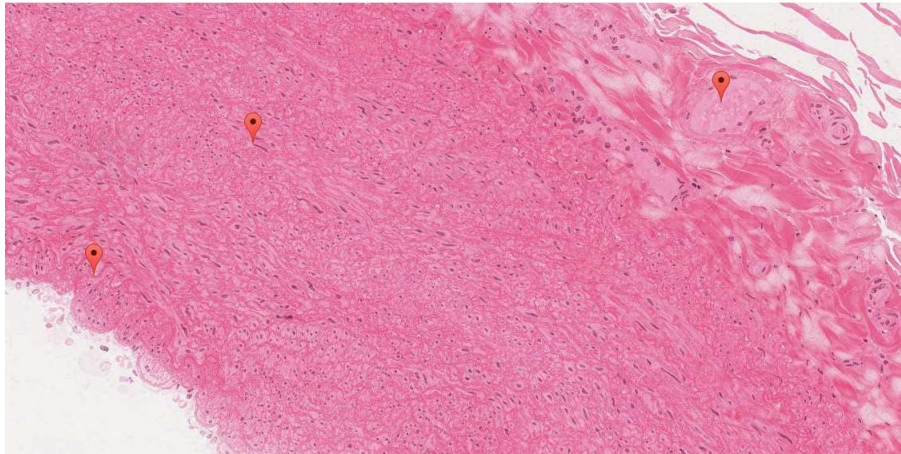


Figure 2.8: Magnification (100x) of a scalloped aortic vessel

http://medcell.med.yale.edu/histology/virtualSlide.php?slide_name=CB502_Histo_0007&lab_name=blood_vessels_lab&slide_id=7

2.8

Belonging to the elastic arteries [5], layers of the ascending aorta walls slightly differ from other arteries such as coronary arteries (muscular arteries) and penicillars (arterioles), in fig. 2.9 layers and connotive elements of the aorta.

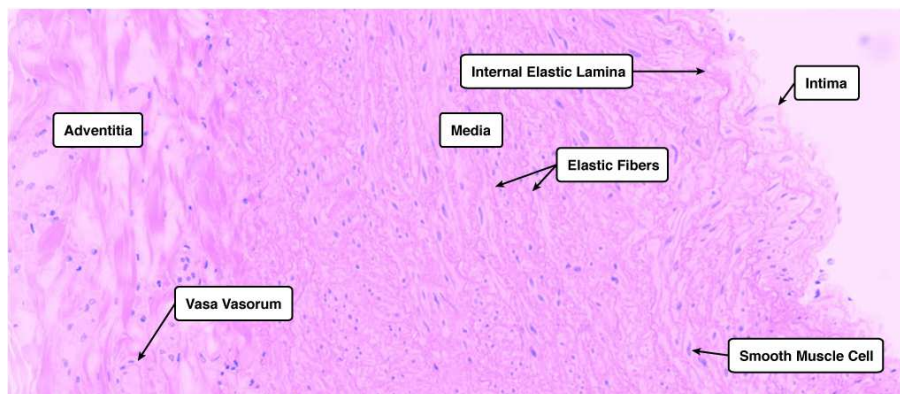


Figure 2.9: Scalloped aortic vessel with labelled elements

http://medcell.med.yale.edu/histology/blood_vessels_lab/aorta_wall.php

2.9

Aortic layers are:

- **Tunica intima** is made up of an epithelium, which is a single layer of flattened epithelial cells supported by a layer of elastin enriched with collagen. Fibroblasts and myointimal cells are also plunged in the epithelium (fig. 2.10). Myointimal cells accumulate lipid with ageing leading to a thickening of the intima layer, one of the first signs of atherosclerosis.

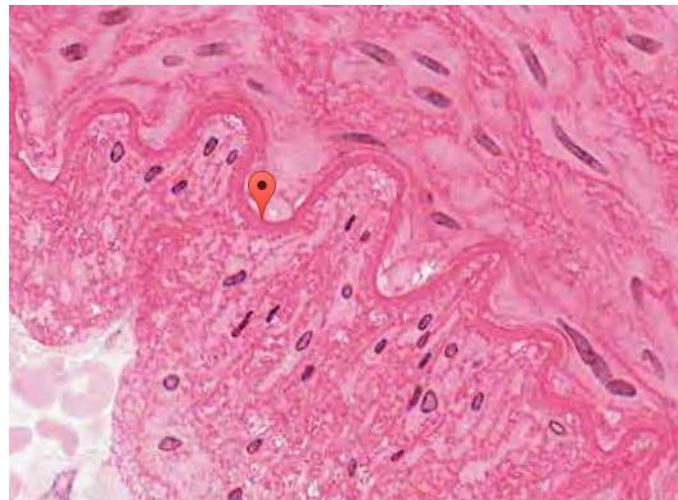


Figure 2.10: Pinpointed in the picture is the internal elastic lamina which divides intima and media tunica, below it are clearly visible endothelial cells and above it smooth muscle cells are present

http://medcell.med.yale.edu/histology/virtualSlide.php?slide_name=CB502_Histo_0007&lab_name=blood_vessels_lab&slide_id=7

2.10

- **Tunica media** with its numerous concentric elastic lamellae interspersed with clusters of smooth muscle cells (fig. 2.11) is characteristic of these vessels. Elastin can be identified with specific stains and all these elastic fibres (also made by fibrillin too) in the media allow the maintenance of blood pressure through the expansion and contraction of the vessel walls (Windkessel effect); the heavy presence of this substance gives, the “elastic” adjective to this group of artery.

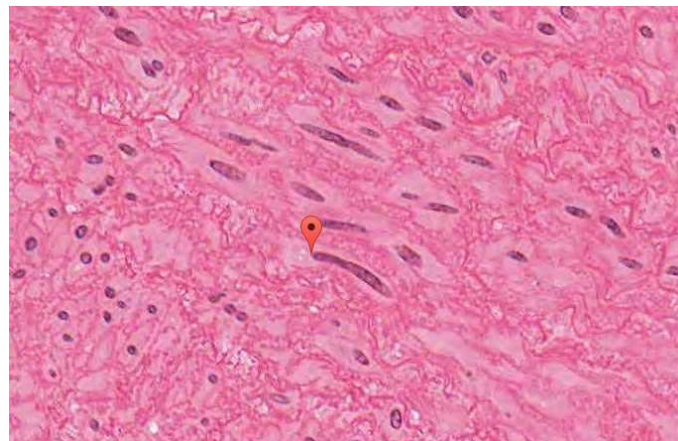


Figure 2.11: Pinpointed in the picture above is a muscle cell of the aortic wall

http://medcell.med.yale.edu/histology/virtualSlide.php?slide_name=CB502_Histo_0007&lab_name=blood_vessels_lab&slide_id=7

2.11

- **Tunica adventitia** and its vasa vasorum (fig. 2.12) network (small vessels that supply the cells of the aorta) are present in this latter layer and outer part of the media because large arteries need their own blood supply.

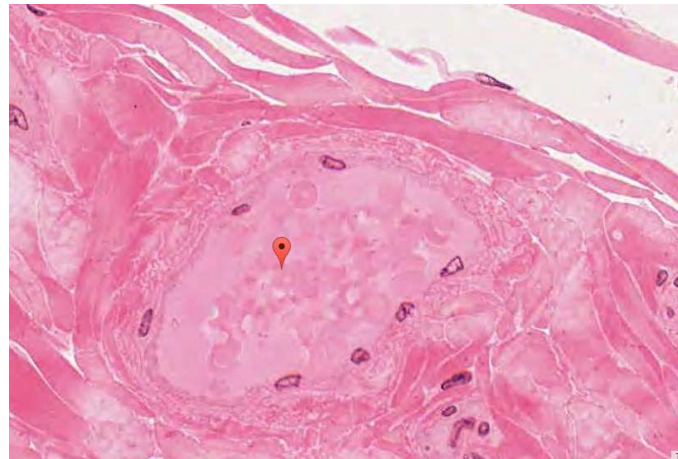


Figure 2.12: In the picture above a clear image of a vasa vasorum delved into the tunica adventitia's connective tissue

http://medcell.med.yale.edu/histology/virtualSlide.php?slide_name=CB502_Histo_0007&lab_name=blood_vessels_lab&slide_id=7

2.12

2.3 Physiology

Apart from being a vessel that distributes oxygenated blood, pumped from the heart to the peripheral circulatory system, aorta has another very important function. Looking at its position in the circulatory system (fig. 2.13), it is the high elasticity which is the main feature of the aortic arch and of the ascending aorta. Because it is the first to receive blood outflow from the heart this vessel is designed to conduct the enormous blood pressures generated by the heart without significant loss to downstream vasculature.

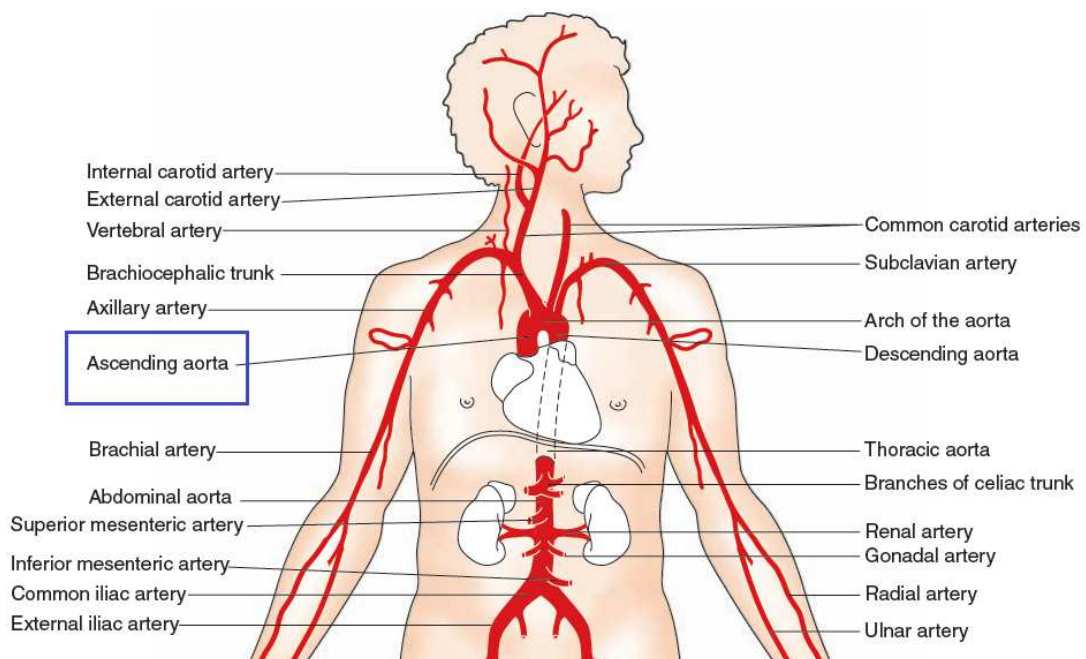


Figure 2.13: Position of AAO in the cardiovascular system [Moini2012]

2.13

Being flexible tubes ^{Beiz1995} [7] they expand, while accepting large volumes of blood from the heart during systole, and rapidly squeeze that blood volume downstream without significant loss of energy. To do so, elastic arteries must be stretchy and possess incredible recoil, which is achieved primarily by large bundles of elastin present in their tunica media (up to 40 per cent in the ascending portion of the aorta). This distention of these vessel make them working as a buffering chamber ^{Bader1983} [8]. Changes in the distensibility are caused by ageing and certain pathological conditions that interfere with the structural nature of the wall, blood flow in the vasa vasorum or a passive stretching of the aorta for high blood pressures ^{Stratosetal1992} [9].

2.3.1 Windkessel effect

Knowing that the heart pumps blood rhythmically (diastole and systole) to the network (fig. ^{2.14} 2.14) how does a continuous blood flow come to be?

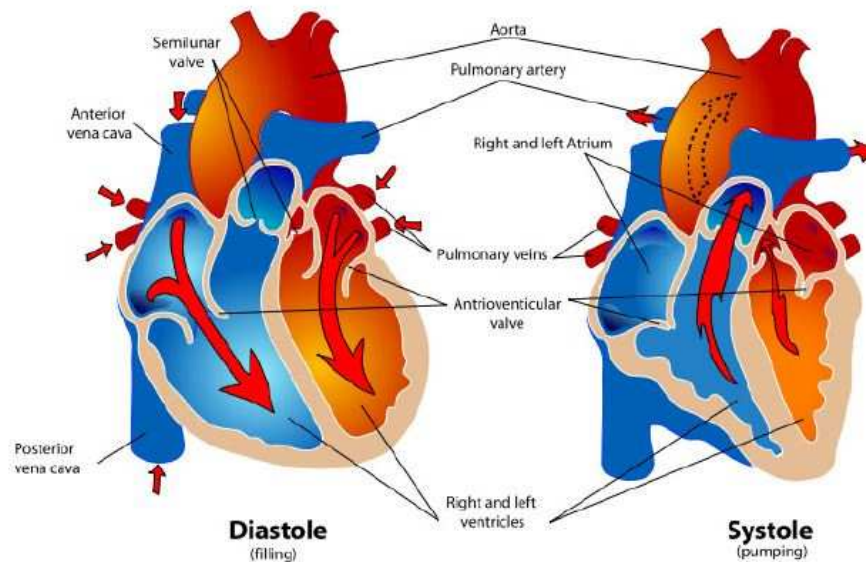


Figure 2.14: The aorta location in the systole-diastole act http://simple.wikipedia.org/wiki/Blue-baby_syndrome

2.14

During systole the blood is pumped out from the left ventricle, under this very high pressure the ascending aorta dilates (fig. ^{2.15} 2.15) due to its high elasticity.

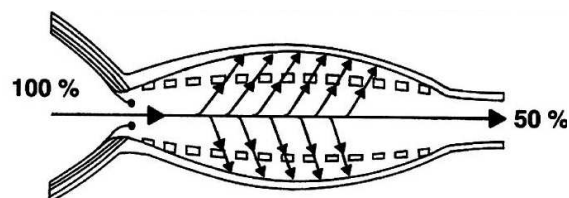
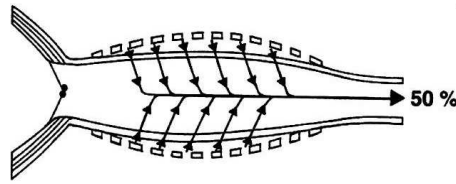


Figure 2.15: Aorta during systole.

2.15

Then diastole makes the aortic valve closing (fig. ^{2.16} 2.16) preventing blood flow. It would not through the entire vessel system but for the aortic wall, without the previous pressure, slowly recoiling and then pushing out the previously stored blood in its last expansion.



2.16

Figure 2.16: Aorta during diastole.

All this results in a nearly continuous peripheral flow, although the heart pumps non continuously with its rhythmic contractions: this described mechanism is called Windkessel effect. The Windkessel effect and this important function of the aorta depend on the elasticity of the aortic wall. With “elastic” is defined any body that can store energy without loss of energy [10]. To explain this effect in simple “energy” terms it can be said that the kinetic energy of blood ejected at systolic stroke is stored within the dilated aortic walls as potential energy which is then reconverted into kinetic for the blood at the diastole with the aortic recoil. With some simplifications it can be said that the elastic resistance (or elastance or stiffness) describes, indeed, the resistance offered to the distention of the aorta itself and is a needed quantity to understand aneurysmal aortic wall mechanical properties this is why its characterising modulus E (Young’s modulus) has been registered through the tensile tests.

Causes of changing in aortic elastic properties

- **Age**

Rise of the blood pressure increases the aortic stiffness because when the distending pressure rises most of the load is borne by the collagenous fibres (less extensible) than the elastin ones ([11], [12], [13], [8], [14]). The pressure rising is due also to ageing. This age-related increase in resistance was (among the others) also confirmed by NMR studies [15].

- **Gender**

Clear dependency of aortic elastic properties on gender has been demonstrated in age groups from twenty to fifty years old; before and after this age window, respectively the not-yet-completed sexual development and menopause/climacteric equalize the two groups [16].

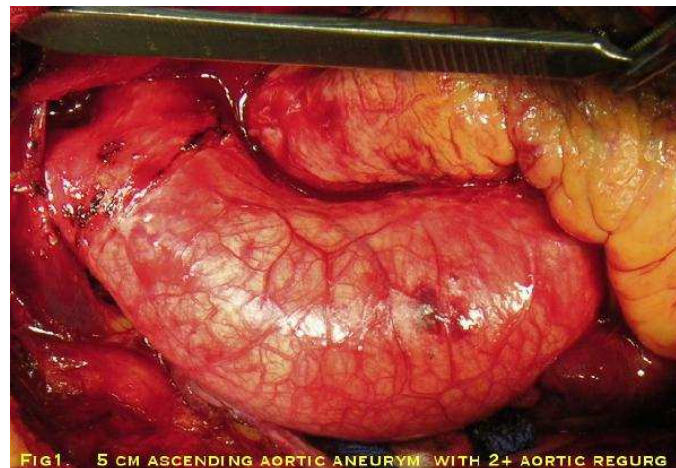
- **Vascular pathology**

Atherosclerosis ([15], [17]), diabetes mellitus and hypercholesterolemia [18] [19], hypertension and all congenital syndromes (such as Marfan’s) have a direct influence.

All of this factors participate in the increasing rigidity of the aorta because, from an histological point of view, a loss of elastic fibres and an increase in collagen tissue [8] change the normal 40 per cent of elastin present in the tunica media.

2.4 Pathologies

The most widespread aortic pathologies are the obstructive and dilative type. In the obstructive type cholesterol forms plates, coagulations that can thicken till the closure of the lumen. In the dilative types the aortic wall collapse in various way leading to aneurysm or dissection. Aneurysm is one of the most striking vascular pathology among all the pathologies of the ascending portion of the aorta . An abnormal localized dilation of a vasal lumen happens and, as it increases in size, the risk of rupture grows exponentially. Its definition is clarifying: Aneurysm is a permanent dilatation (fig. 2.17) of an artery by partial interruption of its elastic and muscular constituents [20].



2.17 Figure 2.17: 5 cm ascending aortic aneurysm https://www.youtube.com/watch?v=h_u6SfPhHyc

Although they can show up in every single blood vessel the most frequent and significant are the aortic ones that, in breaking, result in being fatal.

A macroscopic classification ^{Gallone1969} [20] is categorizing aneurysmal dilatations by their shape (fig. 2.18).

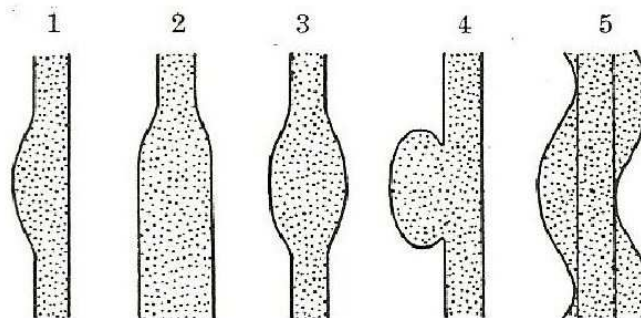


Figure 2.18: Various shapes of aneurysm ^{Gallone1969} [20]

2.18

1. Navicular

They can be named as dissecting aneurysms because they are caused by a dissection of tunica layers.

2. Cilindroid

A particular case of extremely long fusiform aneurysms.

3. Fusiform

This type occurs when there is a gradual dilatation of the vascular lumen to which they are in a direct continuity with their own one. Usually their diameter is 20 cm as a maximum and normally they appear in the ascending portion of the aortic arch (fig. 2.19) and in the abdominal aortic tranche. Being an eccentric dilatation, a portion of the vasal wall results in being more severely damaged.

4. Saccular

A spherical dilatation with a diameter from 5 cm to 10 cm. The aneurysmal sac is connected to the lumen of the vessel by a hole of different sizes which can virtually have the same diameter of the dilatation. Usually there is a thrombus partially or fully covering the aneurysm.

5. Cirroid

Resembling a varix is often caused by trivial trauma.



Figure 2.19: Radio imaging of a fusiform aneurysm in an ascending aorta
<http://radiopaedia.org/images/353528>

2.19

2.4.1 Aetiology and pathogenesis

An aetiological division of aneurysms can be grouping in two main classes: congenital and acquired, which can be sub-divided into traumatic, infectious and atherosclerotic.

- **CONGENITAL**

Here are grouped, as well as the aneurysmal malformation already present at birth, the postnatal aneurysms attributable to congenital weakness of the aortic partition (such as Marfan's Syndrome fig. 2.20).

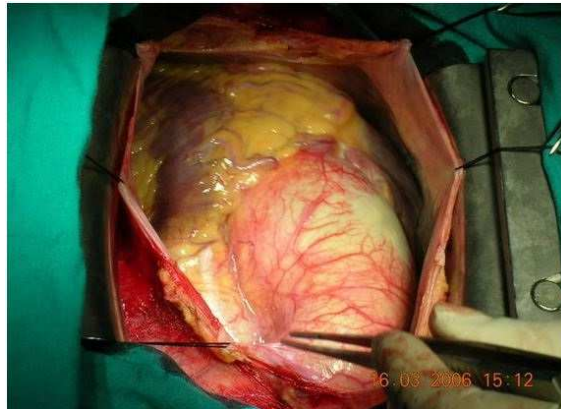


Figure 2.20: Aneurysm of a 18 yo boy suffering from Marfan's Syndrome
<http://www.intechopen.com/books/aortic-aneurysm-recent-advances/aortic-aneurysm-in-children-and-adolescents>

2.20

- ACQUIRED

1. *Traumatic*

Trauma compromising the integrity of the wall vessel can be the trigger cause for a development of an aneurysmal complex. Examples of these could be contusions, partial laceration or compression against bony planes. Aneurysm caused by congenital factors that lead to a higher stress of the wall are grouped in this category, such is an aneurysm caused by a bicuspid aortic valve (fig. 2.21) disease where fused cusps create a bicuspid valve instead of a tricuspid.

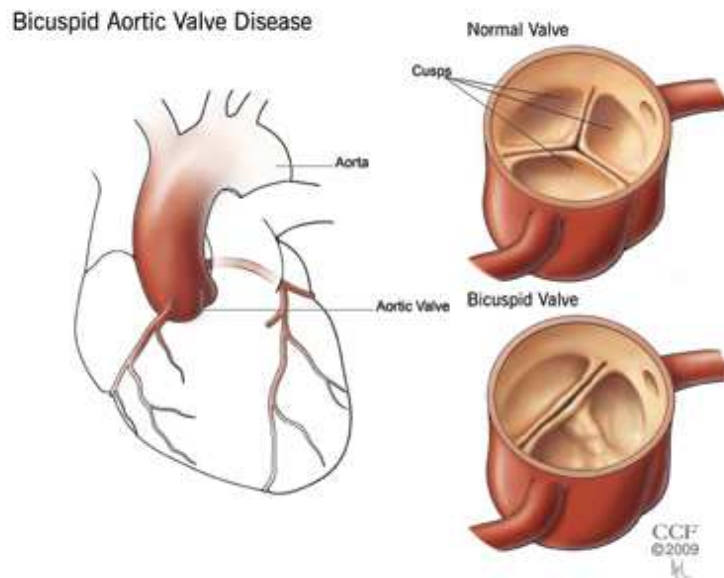


Figure 2.21: Bicuspid aortic valve

2.21

<http://my.clevelandclinic.org/services/heart/disorders/congenital/congenvalve>

This anomaly creates an unusual flux (fig. 2.22) of blood directed straight to the aortic wall instead of the lumen centre line, this continuous force applied on the wall weakens

the latter increasing its probability of aneurysm.

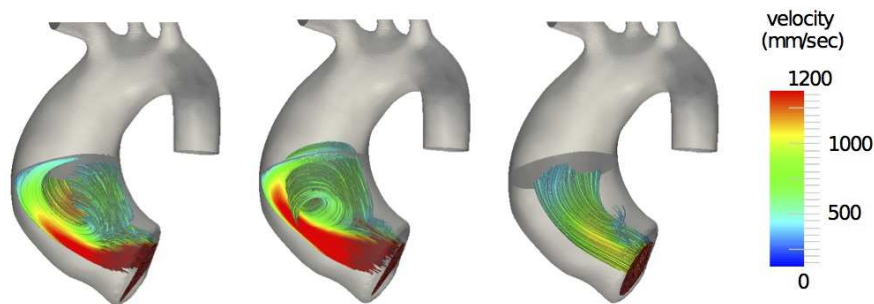


Figure 2.22: Blood flow from an unhealthy, bicuspid valve (on the left and center) and from a healthy, tricuspid valve (on the right)

2.22

<http://mathcard.epfl.ch/page-87341-en.html>

2. Infectious

Syphilis constitutes the most frequent cause of arteritis in aneurysmic evolution which is **often localized in the ascending portion of the aorta** and its arch and this is why this type of arteritis is called aortitis. Spirochaete arrive in the adventitia (fig. 2.23) through vasa vasorum and begin the periaortitis process, from here the infection propagates to the media originating a mesoartitis. This is the cause of the occurrence of an aneurysm because the mesoartitis leads to a gradual destruction of the elastic lamina.

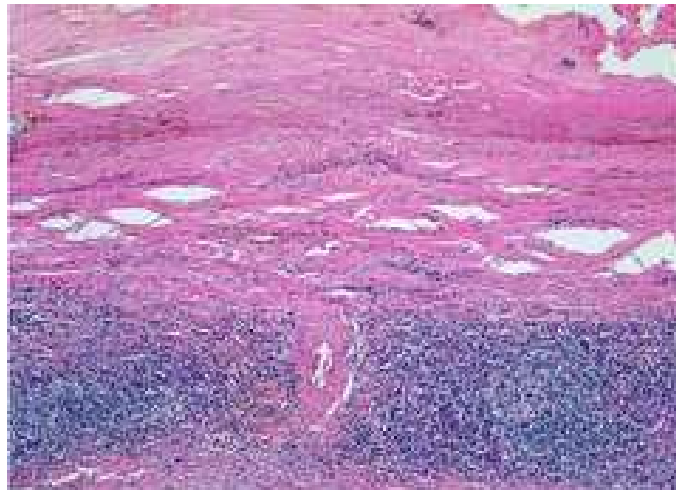


Figure 2.23: Infected adventitia

2.23

<http://quizlet.com/23884591/cardio-lab-flash-cards/>

This can be “classified” as a destruction of the media layer from the outside. The mycotic aneurysms can be put in juxtaposition to the previously discussed. It begins with a thrombotic endoarteritis because of bacterial implants in the tunica intima which later propagate in the media. To be taken into account is that aneurysms of non-bacterial aetiology later become breeding grounds of septic infections. The degenerative alterations of the tunica intima, very likely to happen in the aneurysmatic walls, favour thrombotic deposition which predispose to sporozoan haematogenesis.

3. Atherosclerosis

These aneurysms arise as a consequence of necrobiotic processes that lead the atheroma (fig. 2.24) from the intima to the media (atheroma is an accumulation of degenerative material in the tunica intima of artery walls).

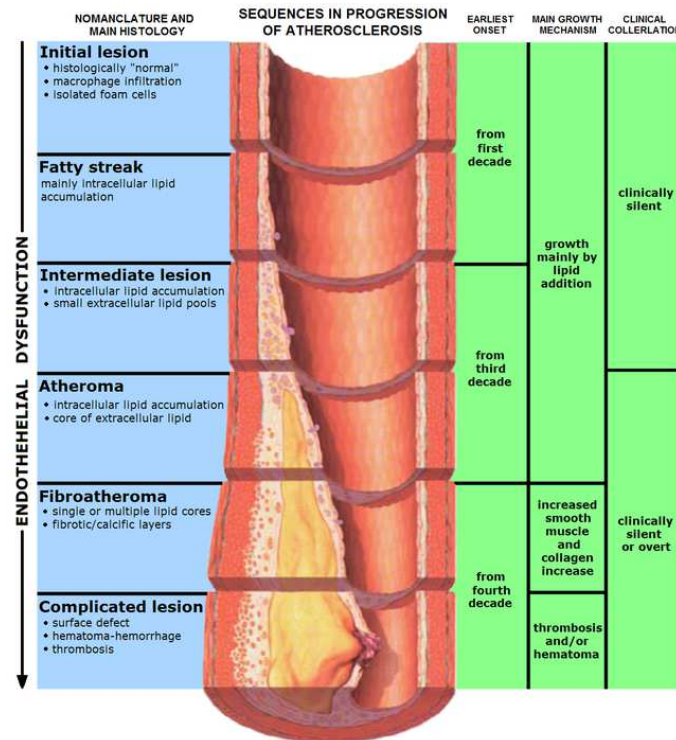


Figure 2.24: Stages of atherosclerosis

<http://en.wikipedia.org/wiki/Atherosclerosis>

2.24

2.4.2 Pathological anatomy

Aetiology clarifies the great variability among anatomic-pathological samples but the main feature remains the destructive phenomenon of media walls [20] which is going to be described. Aneurysmal sac is joined to the contiguous tissues by a dense connective tissue which tends to swallow the adjacent anatomical structures of the neurovascular bundle. The skeletal parts (the sternum for cases of aneurysms of the aortic arch) are subject to osteoclastic deterioration until complete destruction. Circulatory slackening and alteration of the intima contribute to endoaneurysmatic thrombosis that can delay the growth of the aneurysm but, at the same time, brings about ischemic facts and embolism in the peripheral segments. In addition to this regressive alteration, which degrade and wear thin the vasal wall, fibroblastic and angioblastic activities (attempts of restoration) have to be taken into account (fig. 2.25). They are phenomena of scarring scleriosis among destruction of the elastic tissue and muscular cells, with tokens of capillaries that start from the network of vasa vasorum. Furthermore there are attempts of elastic neof ormation favoured by endoarteric pressure, and, in particular, by the rhythmic systolic relaxations which conduct a determinant action for the elastogenesis of the vasal walls regardless of pathological conditions. Tunica intima also partake in this effort to restore the wall with a proliferation of connective and endothelial elements.

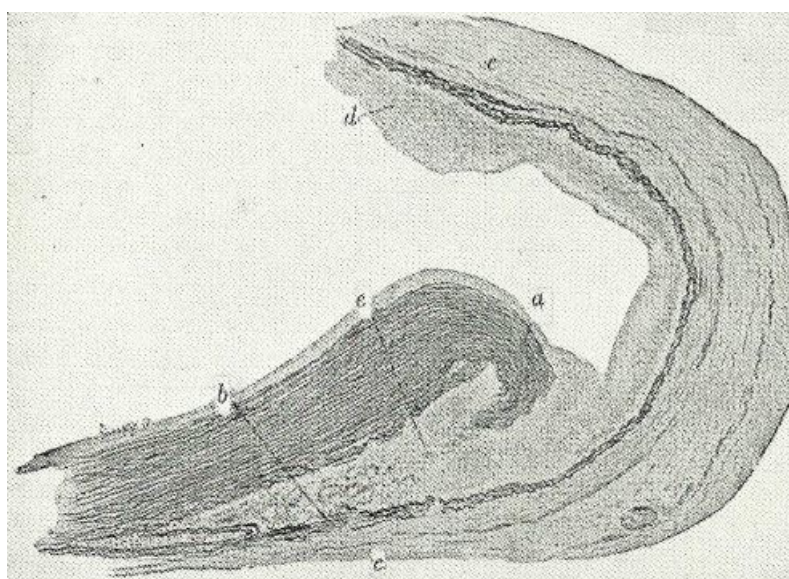


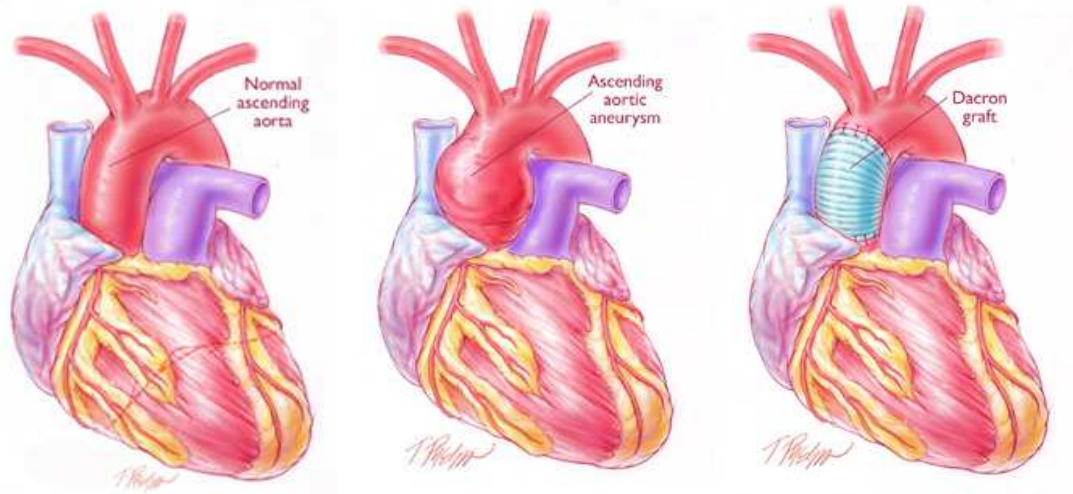
Figure 2.25: Restoration attempt on an aneurysmal aortic wall.

- *a* Intima and media lamina torn and shrunken.
- *b* Conserved external lamina of the tunica media.
- *c* Neo-formed and thickened tunica intima.
- *d* Gap formed by a dissection of lamina of the media but already filled with reparative connective tissue.

2.25

2.4.3 Aneurysm of the ascending aorta

The portion classified as ascending is the first short tract (5 to 7 cm) which originates from the semilunar aortic valve of the left ventricle and ends at the beginning of the aortic arch (fig. 2.26). The coronaries originate from the ascending portion of the aorta. The wall of the aorta, being very elastic, can stretch and then recoil back, as already explained. Because of this, factors shown above have a more serious effect on the ascending aorta than elsewhere additionally because of its vital function; it is not being said that there is a scale of danger in aortic aneurysms but the ascending portion is the one which firstly receives pumped blood from the heart, and the coronary arteries (of which the function is to oxygenate the heart) have their origin here (fig. 2.26b). This means that a rupture here may cause sudden death by serious haemorrhage or a formed clot could break off from the aneurysm and block blood flow to the coronaries. Unfortunately most aortic aneurysms do not cause symptoms except from abdomen, chest or back pain and discomfort that can all come and go or stay constant. If dissection or even rupture is very likely to happen, surgery must be done. The ascending portion is excised and substituted (fig. 2.26c) by a prosthesis of which the surgical method and shape may be variable depending on the position of the aneurysm. These excised portions are what has been studied in this work.



(a) Sketch of a healthy ascending aorta

2.26a

2.26b

(b) Sketch of an aneurismal ascending aorta

2.26c

(c) Sketch of an aneurismal ascending aorta

Figure 2.26: http://www.hopkinsmedicine.org/heart_vascular_institute/conditions_treatments/treatments/aortic_aneurysm_surgery.html

2.4.4 Surgical procedures

procedures

The standard surgical procedures are on today's date ([\[21\]](#), [\[22\]](#), [\[23\]](#), [\[24\]](#))

- **Endovascular surgery** A catheter is inserted into the bloodstream of which the end is the place of attachment of a synthetic graft that is going to be expanded and firmly placed against the wall of the aorta (fig. [2.27](#)) enforcing the weakened vessel and cutting off the blood supply to the aneurysm which shrinks with time.

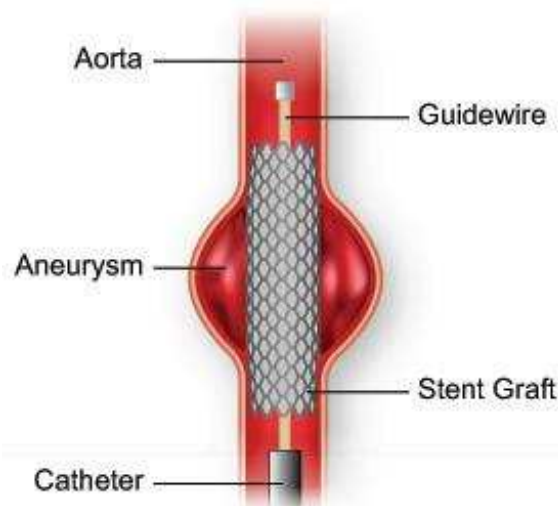


Figure 2.27: A scheme of the insertion of a stent
<http://www.texasheart.org/HIC/Topics/Proced/asurg.cfm>

2.29

- **Open surgery** A replacement of the damaged portion with an artificial graft (fig. [2.30](#)), whose material of composition is Dacron™, sewn with a permanent suture material. For

AsAA, because of its proximity to the aortic valve, the latter can also be repaired or replaced and incisions such as median sternotomy are used.

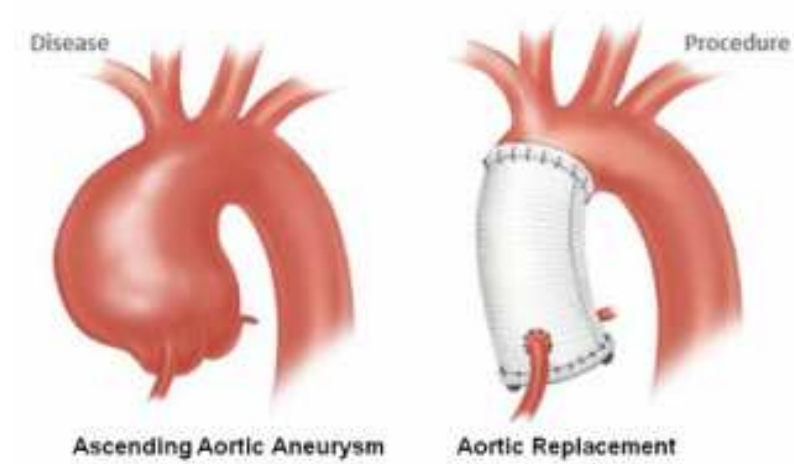


Figure 2.28: A scheme of replacement of the aneurismal portion
<http://www.aortarepair.com/ascending-aortic-aneurysms.html>

Chapter 3

Tensile tests

exp

Portions excised from surgical procedures such the ones described in [2.4.4](#) ^{procedures} have been used in this experiment. On such portions tensile test have been performed. From these, mechanical properties have been recorded in order to characterise and understand the mechanical behaviour of aneurysmal ascending aortic tissue.

3.1 Design, data collection and organization

With these steps a standard method and procedure have been created in order to define a process for uniaxial traction tests of aortic tissues from donors. An established and wide-spread model could help comparisons of statistical tests and of gained informations. Modus operandi has indeed an important effect on statistical results.

3.1.1 Preparation and experimental design

In this chapter are described the methods (from the sample preparation to the data organization) systematically adopted for collecting, preparing and testing specimens of human aneurysm of the ascending aorta.

- **Sample and specimen preparation**

Raw materials used for this experiment: excised portions of ascending aorta aneurysm from 22 y.o. to 82 y.o. patients, who underwent AsAA surgery at IRCCS Policlinico San Matteo di Pavia (IT) between May 2013 and December 2014. Each one has been stored in an isotonic physiological solution at 4°C until mechanical testing and underwent mechanical fatigue within 48 hours from the sample collection. Each sample has been divided into Anterior and Posterior regions (fig. [3.1](#)).



Figure 3.1: Subdivided regions

3.1

Preparation of the single specimens and their mechanical testings have been performed at the Department of Civil Engineering and Architecture of the University of Pavia. First of all connective and adipose tissue lying on the aortic wall have been curretted; then bone-shaped specimens have been cut from both regions.

Each region gave various specimens (whose number depended on each sample size) that have been cut both in longitudinal and circumferential direction as shown in figure 3.2.

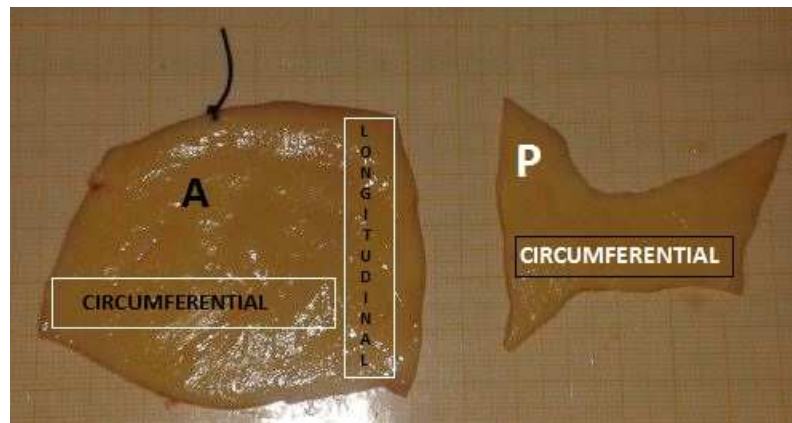


Figure 3.2: Cut aneurysm

3.2

To better quantify and register the mechanical quantities a bone-shaped mould has been adopted because (fig. 3.3) the wider extremities ensure a better grip on the load machine and the thinner central part gives the size standards required for the data harvesting. Specimen extremities have been pasted with super-adhesive glue to patches of sandpaper (to ensure a firm clamping of the tensile-test machine pincers) and marked in the middle part with black lines, the function of which is to gauge mark during the extension measurements.

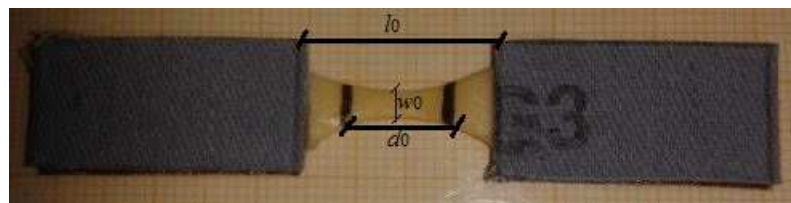


Figure 3.3: Specimen ready to undergo the test

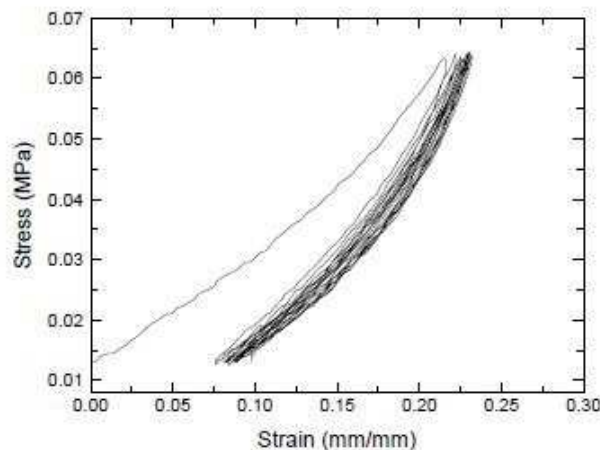
3.3

- **Measured data before tensile test**

Before the tensile test the following quantities have been measured: the initial cross-sectional area A_0 (a product between two quantities: w_0 the initial width of narrowest section and t_0 the initial mean thickness of the narrowest section), the initial distance between markers (d_0) and the initial distance between the grips l_0 .

- **Mechanical preconditioning of the specimen**

To ensure a specimen stabilization, which would have guaranteed repeatable stress-strain curves, 10 cycles in succession of loading-unloading (fig. 3.4) have been effectuated at the same cross-head speed of 10 mm/min with an increasing load from 0.1 N to 0.5 N (frequency of recording 10 Hz).



3.4

Figure 3.4: Representative preconditioning response

3.1.2 Mechanical test machine

In order to perform traction forces on the specimens the MTS Insight Testing Systems 10 kN (MTS System Corporation) has been used (fig. 3.5). Although it can be used for other advanced tests, such as creep, stress relaxation and multi-cycle, it is in its standard performances (peeling, tearing, compressing, flexing/bending and indeed tensile tests) that had been used in this experiment.



Figure 3.5: MTS Insight Testing Systems 10 kN (MTS System Corporation)

3.5

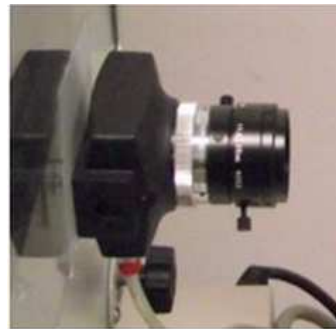
<http://www-2.unipv.it/compmech/mts.html>

It is composed of a double-column load frames and a moving solid steel cross head. The MTS system is equipped with wedge and pneumatic grips for testing hard and soft materials respectively, so the latter have been chosen (fig. 3.6a) for the aortic tissue. A 250 N load cell (fig. 3.6b) anchored to the cross-head detects the applied loads during testing. This was the appropriate load for this type of experiment instead of the other cell of 10kN. The ME-46 Video Extensometer (fig. 3.6c) measures the specimen deformations referring to the markers.



a

(a) Soft tissue grips



b

(b) E-46 video Extensometer



(c) 250 N load cell

Figure 3.6: Three details of the MTS machine

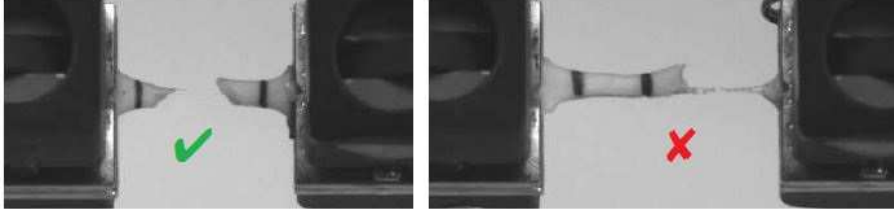
3.6

<http://www-2.unipv.it/compmech/mts.html>

Uniaxial tensile tests

Uniaxial tensile tests have been performed on each bone-shaped specimen at the same cross-head speed of the preconditioning (10mm/min) but this time until rupture of the specimen. Successful tests are the ones where specimen broke in the area included by the two black markers, unsuccessful are the those where rupture point fell outside the markers. This is because it would not be

evaluated by the E-46 Video Extensometer of which the focus is the area enclosed in the marks.



3.7

Figure 3.7: Examples of successful and unsuccessful experiments

3.1.3 Data collection

3.1.3

Data processing and curve fitting

The raw and readily available machine-level measured data are the previously mentioned: A_0 and d_0 ; the load applied F and the extension Δd of the specimen have also been recorded. The latter two were post-processed with Matlab 7.10.0 [25] and *engineering stress* (σ_E) and *engineering strain* (ε_E) have been computed as shown in eq. I :

$$\sigma_E = \frac{F}{A_0} \quad \varepsilon_E = \frac{\Delta d}{d_0} \quad (\text{I}) \quad \boxed{\text{I}}$$

True stress (σ_T) and *true strain* (ε_T) were computed (II) with the current cross-sectional area and distance d instead of their initial equivalent used in the engineering values.

$$\sigma_T = \frac{F}{A} \quad \varepsilon_T = \int_{d_0}^d \frac{\delta d}{d} = \ln \frac{d}{d_0} \quad (\text{II}) \quad \boxed{\text{II}}$$

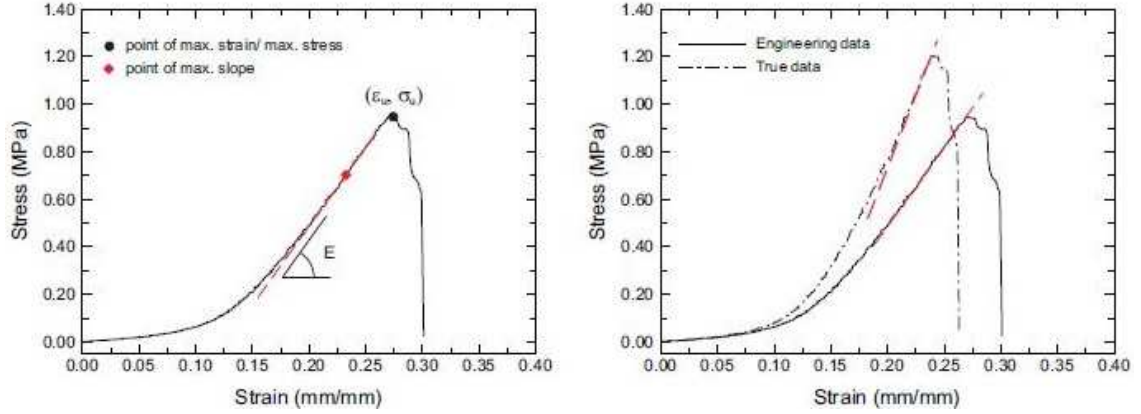
In order to plot the engineering and true strain-stress curves the relation (III) had to be taken into consideration, which bonds strain and stress assuming the material incompressibility.

$$\sigma_T = \sigma_E(1 + \varepsilon_E) \quad \varepsilon_T = \ln(1 + \varepsilon_E) \quad (\text{III}) \quad \boxed{\text{III}}$$

According to Sokolisetal2002 [26] a polynomial function has been used (eq. IV) of the 7th order:

$$\sigma(\varepsilon) = p_0 + p_1\varepsilon + p_2\varepsilon^2 + p_3\varepsilon^3 + p_4\varepsilon^4 + p_5\varepsilon^5 + p_6\varepsilon^6 + p_7\varepsilon^7 \quad (\text{IV}) \quad \boxed{\text{IV}}$$

with p_i -th being stress-like coefficients. Curves have been fitted as in fig. 3.8, from them and quantities of interest (such as peak strain and peak stress) have been extrapolated.



3.8

Figure 3.8: Engineering and true strain-stress curves

Extrapolation of quantities of interest

Four are the mechanical quantities studied in this analysis:

1. **Thickness** t of the aneurysmal aortic wall previously measured with a digital caliper.
2. **Peak strain** ε_U the maximum *strain* reached before breakage which is the maximum sustainable **deformation** before rupture.
3. **Peak stress** σ_U the maximum *stress* reached before breakage which is the maximum sustainable **force** before rupture.
4. **Maximum elastic Young's modulus** E_{MAX} the maximum *Young's module* reached before breakage which is the **stiffness** of the tissue.

The equation of the last (being as its nature the derivative of the stress-strain curve) had to be computed from deriving (V) and then maximizing (VI) the eq.(IV), in formula:

$$E(\varepsilon) = \frac{d\sigma}{d\varepsilon} = p_1 + 2p_2\varepsilon^1 + 3p_3\varepsilon^2 + 4p_4\varepsilon^3 + 5p_5\varepsilon^4 + 6p_6\varepsilon^5 + 7p_7\varepsilon^6 \quad (V) \quad \boxed{V}$$

And then

$$E_{MAX} = \max_{\varepsilon} E\varepsilon \quad (VI) \quad \boxed{VI}$$

Following this the quantities that will undergo statistical analysis have been extrapolated but first they have to be manipulated and organized.

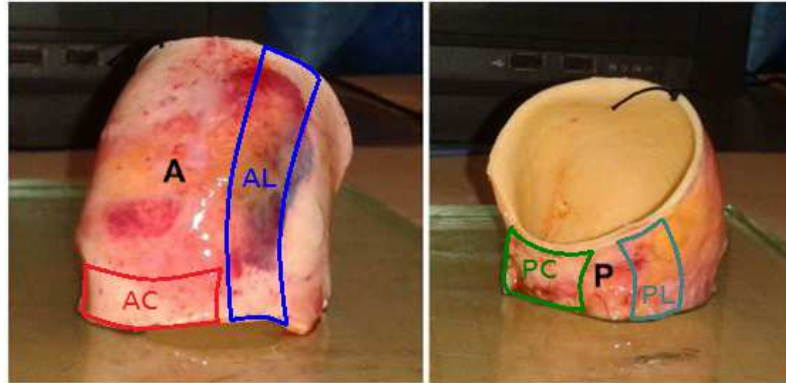
3.1.4 Data organization

3.1.4

Because of failed rupture in the marked zone or for anomalies in the machine/calculator in the data recording not all the specimens gave consistent data to the analysis. Therefore patients are represented in their age, gender (divided into three main classes) categories by single, multiple or even mean values (depending on the statistical analysis under which they will undergo) for each studied quantity in each of the four zones (fig. 3.9) of study (by zone it is meant : *location* on the aortic wall and *direction*).

- Anterior Longitudinal
- Anterior Circumferential
- Posterior Longitudinal

- Posterior Circumferential



3.9

Figure 3.9: Subdivision of the sample

Data tables will be elaborated using R software [27]^{R2014} and will look like this example of the mean data in fig. 3.10:

pat	thicknex	strain.break	peakstrex	modulus	sex	age	category
2	1.800000	0.3415000	0.4740000	3.020000	M	75	e
3	1.600000	1.0470000	0.7570000	4.198000	M	58	m
4	1.300000	0.2540000	0.5070000	8.732000	M	78	e
5	1.550000	0.6590000	0.8060000	3.646000	M	51	m
6	1.260000	0.5130000	1.3280000	7.366000	F	51	m
7	2.450000	0.5590000	0.4380000	2.017000	M	54	m
8	1.300000	0.1840000	0.2900000	2.339000	F	80	e

Figure 3.10: Legend:

- pat stands for *Patient's Identification Number*
- thicknex stands for *Thickness*.
- strain.break stands for *Peak Strain*.
- peakstrex stands for *Peak Stress*.
- modulus stands for *Maximum elastic Young's modulus*.

3.10

Chapter 4

Mathematical tools

maths

4.1 Statistical Methods

The collected and organized data shown in chapter ^{exp}3 are now ready to undergo statistical analysis via R software, in this chapter a brief illustration of the statistical methods used in the analysis and how R performs them (the overall logic) has been presented.

4.1.1 ANOVA

ANOVA or ANalysis Of VAriance is a procedure used to test hypotheses about means in several population situations. This technique tests the hypothesis that means among two or more groups are equal, assuming the normal distribution of the sampled populations. It answers the questions: Is there a difference between groups?

Is there a role played by variables on the studied effect?

Example

The object of the study is the effect of moistness on the rate of growth of lichens. A rate of growth is calculated before placing n cultures of lichens each in three different greenhouses; each greenhouse is at a selected level of humidity and after 3 days responses are analysed, in other words we analyse differences of the rate of growth between before and after being subjected to the humidity. Humidity is a *factor* that has been set to certain percentages so, in this example, there are three levels or settings of the humidity factor.

FACTOR: A factor is an independent treatment variable of which the values are controlled and varied by the experimenter.

LEVEL: Levels can be quantitative numbers or categorical data.

ANOVA can be differentiated basing on the number of factor partaking in the analysis:

- **One way ANOVA** The example shown presents only the humidity factor, the analysis of variance will be called one-way/factor ANOVA.
- **Two/Three way ANOVA** If levels of nutriment were also administrated to lichens there would be two factors: Humidity and Nutriment, this would have lead the technique to the two-way/factor ANOVA. Another factor would, clearly, result in a three-way ANOVA and so on.

Even if more factors are added to the study (leading to an *n-way* ANOVA) the subject of testing still remains the same: hypotheses on the equality of means. Therefore the **Null Hypothesis** of ANOVA is formulated: there is **no difference** in the population means of different levels of humidity factor (called for simplicity level A). The alternative hypothesis is consequently the opposite: there is **difference** between means, they are not equal.

With reference to the two-way ANOVA, calling humidity “factor A” and nutriment “factor B”, the null hypotheses to be tested are:

- No difference exists in the means of factor A
- No difference exists in the means of factor B
- No interaction exists between factors A and B

Alternative hypothesis are:

- Difference exists in the means of factor A
- Difference exists in the means of factor B
- Interaction exists between factors A and B

One-way ANOVA

The basic concept of this procedure is splitting total variation among data into portions: portion due to random error and other portions due to changes in the independent variables ^{anovanist} [28]. It can be argued why t-test, in pairs, could not be done; the reason is that, even in the best situation (independent t-tests) each one makes a mistake with a probability of $(1 - \alpha)^n$. Actually they are not even independent making situation even more complicated and subjected to error. Fisher introduced a revolution in statistics with the ANOVA procedure: instead of studying one single factor in homogeneous groups, many factors are simultaneously studied.

The variance of n samples (measurements) is calculated as follows

$$s^2 = \frac{\sum_{i=1}^n (y_i - \bar{y})^2}{n - 1} \quad (1) \quad \boxed{1}$$

where

- \bar{y}
Is the *mean* of the n samples
- $\sum_{i=1}^n (y_i - \bar{y})^2$
Is the *sum of squares* of deviations from the mean
- $(n - 1)$
Are the *degrees of freedom*

Variance can be rewritten as:

$$s^2 = \frac{\sum_{i=1}^n y_i^2 - \frac{1}{n} (\sum_{i=1}^n y_i)^2}{n - 1} \quad (2) \quad \boxed{2}$$

where

- $\sum_{i=1}^n y_i^2$
Is the *raw sum of squares*
- $\frac{1}{n} (\sum_{i=1}^n y_i)^2$
Is the *sum of squares* of deviations from the mean

The numerator is what is used in the ANOVA technique, called *corrected sum of squares*, is usually referred as **SS** or **SS(Total)** and it can be split into two: *sum of squares of treatments*, or **SST**, and *sum of squares of error*, or **SSE**.

$$\mathbf{SS(Total)} = \mathbf{SST} + \mathbf{SSE}$$

In formula

$$\sum_{i=1}^k \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_{..})^2 = \sum_{i=1}^k n_i (\bar{y}_{i.} - \bar{y}_{..})^2 + \sum_{i=1}^k \sum_{j=1}^{n_i} (y_{ij} - \bar{y}_{i.})^2 \quad (3) \quad \boxed{3}$$

where:

- k
is the number of *treatments*
- $\bar{y}_{..}$
is the *overall mean*
- n_i
is the number of observation for *i-th* treatment
- N
is the total number of observations

Treatment A treatment is a specific combination of factor levels, the effect of which is to be compared with other combinations or, indeed, treatments.

Mathematical model and assumptions The fixed effects model is that which is used in this statistical test, the existing bond between response, the dependent variable, treatment and the independent variable, (for the one-way ANOVA) is described by the following formula:

$$Y_{ij} = \mu + \tau_i + \epsilon_{ij} \quad (4) \quad \boxed{4}$$

where:

- Y_{ij}
Is the *j-th* observation ($j = 1, \dots, n_i$) on the *i-th* treatment ($i = 1, \dots, k$ levels)
- μ
Is the communal effect or *overall mean*
- τ_i
Is the *i-th* treatment effect
- ϵ_{ij}
Is the random error present in each *j-th* observation of the *i-th* treatment or the *residual casual factor* which is the unexplained variability

Assumption of the fixed effects model

- ϵ_{ij}
Errors are assumed to be **NID** : Normally and Independently Distributed with a null mean and variance σ_ϵ^2
- μ
Is a fixed parameter and chosen so that: $\sum \tau_i = 0$ where $i = 1, \dots, k$

- τ_i
Are fixed parameters if the levels of treatment are fixed (which is this case of study). As written above, being the overall mean fixed μ , their sum gives zero.

If the k levels of treatment are chosen at random it would be a random effects model.

Computation of the statistic **SST** and **SSE** being, respectively, the total sum of squared deviations from the overall mean and the total sum of the squared deviations within groups, they play an important role in the calculation of the used statistic. They are used to compute two **mean squares**, for respectively *treatments* (**MST**) and error (**MSE**), simply dividing them by the corresponding degrees of freedom (**DFT** for treatments and **DFE** for error). N being the total number of observations degrees of freedom are calculated:

$$DFT = k - 1$$

$$DFE = N - k$$

So the matching mean squares (under the model hypothesis) are a non-distorted valuation of the variance and they are calculated as follows:

$$MST = SST/DFT$$

$$MSE = SSE/DFE$$

Finally the test statistic is computed:

$$F = MST/MSE$$

If the null hypothesis of equal means is true, the mean squares estimate the same quantity : namely the error variance so they should be of approximately equal magnitude (*ratio* ≈ 1). If the null hypothesis of equal means is false then **MST** should be greater than **MSE**.

Being the statistic **F** distributed as a Fisher-Snedecor distribution the critical value is decided on the tabular values of this distribution at the chosen level α and at the degrees of freedom: **DFT** and **DFE**. It must be noticed that this is a unilateral test because the statistic **F** is always greater than 1.

ANOVA table shows results as follows:

Source	DF	SS	MS	F
Treatments	k-1	SST	SST/(k-1)	MST/MSE
Error	N-k	SSE	SSE/(N-k)	

Having calculations done with R ^{R2014} [27], it outputs an ANOVA table with an additional column displaying the p-value of the respective computed F statistic.

Example

Recalling the lichens experiment, differences in the rate of growth from subjecting lichens, coming from same parents, to three different levels of humidity for a period of 3 days have been measured on samples of five elements (one sample per each group). So differences have been measured from an experiment in which each of three treatment was replicated 5 times.

1 Level	2 Level	3 Level
6.9	8.3	8.0
5.4	6.8	10.5
5.8	7.8	8.1
4.6	9.2	6.9
4.0	6.5	9.3

In step the manual procedure is as follows:

1. Computing CM , the Correction for the Mean.

$$CM = \frac{(\sum_{i=1}^3 \sum_{j=1}^5 y_{ij})^2}{N_{total}} = (Total\ of\ all\ observations)^2 / N_{total} = \frac{(108.1)^2}{15}$$

2. Computing total SS .

$$SS(Total) = \sum_{i=1}^3 \sum_{j=1}^5 y_{ij}^2 - CM = 6.9^2 + \dots + 9.3^2 - CM = 829.39 - 779.041 = 45.439$$

3. Computing SST .

By doing this the total sum for each treatment has to be calculated

$$T_1 = 6.9 + 5.4 + \dots + 4.0 = 26.7$$

$$T_2 = 8.3 + 6.8 + \dots + 6.5 = 38.6$$

$$T_3 = 8.0 + 10.5 + \dots + 9.3 = 42.8$$

Therefore,

$$SST = \sum_{i=1}^3 \frac{T_i^2}{n_i} - CM = \frac{26.7^2}{5} + \frac{38.6^2}{5} + \frac{42.8^2}{5} - 779.041 = 27.897$$

4. Calculate MST , MSE and then their ratio F to finally end the procedure.

$$MST = \frac{SST}{k-1} = \frac{27.897}{2} = 13.949$$

$$MST = \frac{SSE}{N-k} = \frac{17.452}{12} = 1.454$$

$$F = \frac{MST}{MSE} = 9.59$$

5. Draw the ANOVA table.

Source	DF	SS	MS	F
Treatments	2	27.897	13.949	9.59
Error	12	17.452	1.454	

F value is 9.59, with the commonly-used 0.05 value of threshold (or previously termed α) and

degrees of freedom, looking at the F distribution table a 3.89 is showed in correspondence of these three elements (α , **DFT**, **DFI**). Being the test statistic much larger than the value seen in the table, the null hypothesis can be rejected and statistically conclude that there is a difference among the population means. The p-value for 9.59 (reviewing F-table) is of 0.00325, so the conclusion is significant at that level.

IMPORTANT: It has statistically been proven that there is a difference among the three means but it is not known, at this point, whether the three means are all dissimilar or which of the three is the different one and by how much it is different.

Two-way ANOVA

Approaching the two-way ANOVA the definition of a *factorial experiment* is needed:

An experiment utilizing all the combinations of factor levels as treatments.

In a two-way ANOVA, two factors (**A** and **B**) with, respectively, a and b levels, have to be taken into account while forming the general model of the effects:

$$Y_{ij} = \mu + \tau_i + \beta_j + \gamma_{ij} + \epsilon_{ijk} \quad (5) \quad \boxed{5}$$

for
 $i = 1, 2, \dots, a - 1, a$
 $j = 1, 2, \dots, b - 1, b$
 $k = 1, 2, \dots, r - 1, r$
 where:

- Y_{ij}
Is the j -th observation ($j=1, \dots, n_i$) on the i -th treatment ($i=1, \dots, k$ levels).
- μ
Is the communal effect.
- τ_i
Is the i -th level of factor **A**.
- β_j
Is the j -th level of factor **B**.
- γ_{ij}
Is the interaction between the i -th level of **A** and the j -th level of **B**.
- ϵ_{ijk}
Is the error.

With **A** and **B** as *fixed factors* the model is a *fixed-effects model* and the factorial experiment has an equal number of observations per treatment combination then the total sum of squares is as follows (**AB** represents the interaction between the two factors):

$$SS(Total) = SS(A) + SS(B) + SS(AB) + SSE$$

where

- $SS(A) = rb \sum_{i=1}^a (\bar{y}_{i..} - \bar{y}_{...})^2$
- $SS(B) = ra \sum_{j=1}^b (\bar{y}_{.j.} - \bar{y}_{...})^2$

- $SS(AB) = r \sum_{j=1}^b \sum_{i=1}^a (y_{ij\cdot} - \bar{y}_{i\cdot\cdot} - \bar{y}_{\cdot j\cdot} + \bar{y}_{\dots})^2$
- $SSE = \sum_{k=1}^r \sum_{j=1}^b \sum_{i=1}^a (y_{ijk} - \bar{y}_{ij\cdot})^2$
- $SS(Total) = \sum_{k=1}^r \sum_{j=1}^b \sum_{i=1}^a (y_{ijk} - \bar{y}_{\dots})^2$

The **ANOVA table** develops itself into:

Source	SS	DF	MS
Factor A	SS(A)	(a-1)	SS(A)/(a-1)
Factor B	SS(B)	(b-1)	SS(B)/(b-1)
Interaction	SS(AB)	(a-1)(b-1)	SS(AB)/((a-1)(b-1))
Error	SSE	(N-ab)	SSE/(N-ab)

`anova_table`

Testing the null hypothesis (previously described):

- No difference exists in the means of factor A.
- No difference exists in the means of factor B.
- No interaction exists between factors A and B.

The **F** statistics (three, one for each) will be computed as for the one-way ANOVA dividing each Mean Square (**MS(A)**, **MS(B)**, **MS(AB)**) for the the Mean Square of the error (**MSE**). All of the calculated statistics are still distributed as a Fisher-Snedecor distribution. The critical value to compare them with is, again, decided on the tabular values of this distribution at the chosen level α and at their respective degrees of freedom. It remains a unilateral test because the statistics is always greater than 1.

Observations

More factors?

Increasing factors rarely help into making Mean Squares more significant. They certainly diminish the error deviance but degrees of freedoms are diminished too so higher values of ratios (calculated as *F* statistics) are requested to be significant. **So a factor has to be added only if it explains an important part of the variability.** Only a posteriori this can be valued with the E.R, "relative efficiency ratio", of which the meaning is: "to obtain the same power in the two methods (the one with more factors and the one with less factors) in the second method are needed E.R.-times data with the first method."

How to quantify the treatment effect?

To quantify the effect an index is used, the coefficient of determination R^2 which can be:

- A non corrected one (with no degrees of freedom taking part in the calculus), which is simply a ratio between the explained deviance and the total deviance computed as:

$$R^2 = \frac{SS_{exp}}{SS_{tot}} = \frac{SS_{reg}/n}{SS_{tot}/n} \quad (6) \quad \boxed{6}$$

- A corrected one (with degrees of freedom playing a role) computed as:

$$\bar{R}^2 = 1 - \frac{SS_{exp}/df_e}{SS_{tot}/df_{tot}} \quad (7) \quad \boxed{7}$$

Assumptions that have to be verified in order to have credible results of the ANOVA are:

1. Independence of the samples.
2. Normality of the data distribution.
3. Homoscedasticity.

While the independence of samples has a practical effect on the collection of data the last two conditions have to be verified with statistical tools, they can vary in difficulty and empirism, from plots to statistical tests and in this work tests co-assisted by plots it had been selected. Many tests are available, for reasons of exposition only, the ones used in this work are going to be shown in detail.

4.1.2 Shapiro-Wilk's test

The Shapiro-Wilk's test ^{shapiro} [29] tests the **null hypothesis** that the samples came from a normal distribution. This means that if p -value ≤ 0.05 , then the null hypothesis that the samples came from a normal distribution would have been rejected. To check whether a sample x_1, \dots, x_n came from a normally distributed population Shapiro-Wilk's uses the statistic W computed as shown in the eq. (8):

$$W = \frac{(\sum_{i=1}^n a_i x_i)^2}{\sum_{i=1}^n (x_i - \bar{x})^2} \quad (8) \quad \boxed{8}$$

where:

- $x_{(i)}$ Is the i -th order statistic, i.e., the i -th smallest number in the sample
- $\bar{x} = (x_1 + \dots + x_n)/n$ Is actually the sample mean
- a_i Are constants calculated as:

$$(a_1, \dots, a_n) = \frac{m^T V^{-1}}{(m^T V^{-1} V^{-1} m)^{1/2}}$$

where $m = (m_1, \dots, m_n)^T$ is the transposed vector of expected values of the order statistics of independent and identically distributed random variables sampled from the standard normal distribution and V is the covariance matrix of those order statistics.

Normality tests like Shapiro's test are null hypotheses tests AGAINST the assumption of normality so, virtually, they are no value to the data analyst. When the sample size is small, even large departures from normality are not detected, and when sample size is large, even the smallest deviation from normality will lead to a rejected null: so it is about compromising. It would be much better if one tested the NULL hypothesis that the samples do not come from a Normal distribution, even if the side-effect of this hypothesis testing is that this rare chance does happen very rarely. It must be remembered that rejecting a NULL hypothesis is not the same as accepting the alternative hypothesis. It may be also questioned why not using the well-known Kolmogorov-Smirnov test for testing normality, unfortunately this R function is not suited to test deviation from normality; it can only be used only to compare different distributions. ^{Razali et al 2011} [30] conclusions about best power for a given significance (in order: Shapiro-Wilk, Anderson-Darling, Lilliefors and Kolmogorov-Smirnov tests), it has been decided to use the most powerful the Shapiro-Wilk's test (see Appendix ^{7.1.2} for results) assisted by quantile-quantile plots.

4.1.3

4.1.3 Levene's test

Levene's test is used to test if k samples have equal variances ^[Levenenist 31], or, using an adjective, homoscedastic. ANOVA assumes that variances are equal across groups or samples so the Levene's test is used to verify that assumption. Being an alternative to Bartlett's test it has been chosen because of its minor sensitivity to departures from normality.

Even if the Shapiro-Wilk's test gave strong evidence of samples coming from a normal distribution (Bartlett's test has better performance with this condition) it has been chosen to adopt the Levene test because of the different numerous among samples.

The test statistic W , is defined as follows:

$$W = \frac{(N - k) \sum_{i=1}^k N_i (Z_{i.} - Z_{..})^2}{(k - 1) \sum_{i=1}^k \sum_{j=1}^{N_i} (Z_{ij} - Z_{i.})^2} \quad (9) \quad \boxed{9}$$

where

- W Is the result of the test
- k Is the number of different groups to which the sampled cases belong to
- N Is the total nuber of cases in all groups
- N_i Is the number of cases in the i -th group
- Y_{ij} Is the value of the measured variable for the j -th case from the i -th group

$$Z_{ij} = \begin{cases} |Y_{ij} - \bar{Y}_i|, & \bar{Y}_i \text{ is the mean of the } i\text{-th group} \\ |Y_{ij} - \tilde{Y}_i|, & \tilde{Y}_i \text{ is a median of the } i\text{-th group} \end{cases}$$

- $Z_{i.} = \frac{1}{N_i}$ Are the group means of the Z_{ij}
- $Z_{..} = \frac{1}{N} \sum_{i=1}^k \sum_{j=1}^{N_i} Z_{ij}$ Is the overall mean of the Z_{ij}

The three choices for defining Z_{ij} determine the robustness and power of Levene's test or, in other words: the ability of the test to not falsely detect unequal variances when the underlying data are not normally distributed and the variables are in fact **equal** (robustness), the ability of the test to detect unequal variances when the variances are in fact **unequal** (power). Levene's originally only proposed the use of the mean when Brown and Forsythe ^[BrownForsythe1974 32] extended to use either the median or the trimmed mean in addition to the mean. Using the mean provided the best power for symmetric, moderate-tailed, distributions.

In this study it has been chosen the mean because of the normality assurance given by the Shapiro-Wilk's previously tested upon data samples and the moderate-tailed observed distributions (see Appendix ^[7.1.1] and Appendix ^[7.1.2]).

The significance of W is tested against $F(\alpha, k - 1, N - k)$ where F is a quantile of the F-test distribution, with $k - 1$ and $N - k$ its degrees of freedom, α is the chosen level of significance of 0.05.

Even if ANOVA testing can be sufficient in leading to conclusions another extension of the Analysis of Variance test can be rather helpful.

4.1.4 MANOVA

4.1.4

To understand MANOVA its name can help, in fact the Multiple ANalysis OF VAriance already shows how MANOVA can be seen as a Multiple ANOVA without great differences. It is always better to recall the basis and the simple problems to develop a good understanding of this procedure, so from the very simplest problem:

1. T-test
Its purpose is to understand if *two means* for *two groups* are taken from the same sampling distribution of means.
2. ANOVA
Is the T-test correspondent, except that the *two means*, of which origins are to be studied, are from *two or more groups*.
3. Hotelling's T^2
A multivariate T-test, of which calculus tests whether *two vectors of means* for *two groups* are sampled from the same sampling distribution of means.
4. MANOVA
It can be defined as an extension of Hotelling's T^2

MANOVA: tests whether *two or more vectors* of means for *two or more groups* are sampled from the same (multivariate) sampling distribution of means [33]. MANOVA should be primarily used to test several correlated dependent variables with a single, overall, statistical test. It also should be used to understand how independent variables influence response on the dependent ones. MANOVA, like ANOVA, tells if the mean vectors for several groups differ **but** does NOT tell which variables are causing this difference. To understand MANOVA, a path from ANOVA and recalls of its procedures and principles will be followed.

Example:

The lichens example will be used again to approach practically the theoretical problems and concepts.

The outcome measured variables will be: softness, width and thickness. High values of them all indicate good environmental conditions. The experiment is still the same: a factorial 2x2 design with humidity and nutrients as respectively, first and second factor. Each one has only two levels: high and low. Having equal number of lichens in all four conditions it is assumed, for simplicity, to be a balanced design.

Data example:

Lichen	Humidity	Nutriments	Softness (Pascal)	Width(Inches)	Thickness (Inches)
1	low	HIGH	2	2	0.1
2	HIGH	low	3	1	0.1
.
.
.
n

As previously stated, an univariate ANOVA on each measure contains five effects: Communal effect, humidity effect, mean nutrients effect, interactive effect of both factors and error:

$$Y_{ij} = \mu + \tau_i + \beta_j + \gamma_{ij} + \epsilon_{ijk}$$

MANOVA contains the same. At first sight, MANOVA seems to have all ANOVA's properties and this is completely true. Difference lies in the fact that ANOVA works on a (1x1) mean vector for each group while MANOVA computes with a (px1) vector for any group (where p, being the dependent variables, is three in the lichen's example).

Recalling the fact that ANOVA partitions variance into a part due to error (**err**) and a part due to the factors (**H,N**) then their interaction(**H*N**), in this example can be written as follows:

$$Var_{tot} = Var_H + Var_N + Var_{H*N} + Var_{err}$$

MANOVA, in a multivariate environment, partitions a (p x p) *covariance matrix* in the exact same way: a part due to error, a part due to factors (one per each) and a part due to their interaction; same formula but with a matrix notation.

$$\mathbf{Var}_{tot} = \mathbf{Var}_H + \mathbf{Var}_N + \mathbf{Var}_{H*N} + \mathbf{Var}_{err}$$

In the example all the covariance matrices will be a (3x3).

Matrix structure For example the structure of the covariance matrix of error is displayed, others follow this:

$$\mathbf{Var}_{err} = \begin{matrix} & \begin{matrix} Softness & Width & Thickness \end{matrix} \\ \begin{matrix} Softness \\ Width \\ Thickness \end{matrix} & \begin{pmatrix} \mathbf{V}_{e_1} & cov(e_1, e_2) & cov(e_1, e_3) \\ cov(e_2, e_1) & \mathbf{V}_{e_2} & cov(e_2, e_3) \\ cov(e_3, e_1) & cov(e_3, e_2) & \mathbf{V}_{e_3} \end{pmatrix} \end{matrix}$$

\mathbf{Var}_{err}

Diagonal elements, the error variances, have the same meaning of the ANOVA's ones: an average variability within groups. The error variance can be seen as the mean squares within groups doing a univariate ANOVA for one factor, indeed V_{e_1} is the very number as in the corresponding univariate analysis. Off diagonals elements, on the contrary, can be seen as covariance within groups that, referring for example to the $cov(e_1, e_3)$ inform if lichens within a group that have high values of width also tend to have high values of thickness. In fact covariance can be scaled to correlation and ease the interpretation.

The line of reasoning is by now clear so the others matrices are quickly analysed:

$$\mathbf{Var}_H$$

As each one has its analogue in the univariate ANOVA, this matrix has the correspondent variance in Softness due to Humidity, the same as the one calculated from the univariate ANOVA of the Softness, in its first diagonal element. In its second diagonal element Width variance is the same as the one calculated from an univariate ANOVA. In its third diagonal element Thickness variance, again, the same as the one that could have been calculated from an univariate ANOVA.

$$\mathbf{Var}_{H*N}$$

As previously reasoned it can be said that, for example, the third diagonal element is the variance of Thickness as calculated from an univariate ANOVA.

Off diagonals elements of this matrices, having understood the reasoning, have to be interpreted as *between group variances*. So $cov(1, 2)$ in the Var_H tells whether the Humidity group with the highest mean on the Softness score has the highest on the Width. Again, $cov(2, 3) = cov(Width, Thickness)$ in the Var_{H*N} matrix informs whether groups having high average records on Width also tend to have high average Thickness records.

Recalling ANOVA To better understand MANOVA a quick review of ANOVA is needed for later make conceptual bridges between their respective quantities and reasoning.

It has already been said that ANOVA analyzes the variance therefore it tries to obtain two different estimates of population variance:

First one is a variance-within-groups based variance.

Second one is a variance-of-the-groups-means based variance.

Example:

Four independent groups, equal sizes n , only a single variable has been studied and therefore measured.

Null hypothesis: equal means between groups, that in other words can be written as “all the measures have been sampled from a single normal distribution of mean μ and variance σ^2 .”

So expected values of means and variances of all groups should be the ones of the sampling normal distribution hypothesized above (greek letters). Being samples, each one will have its own mean and variance (latin letters).

Null hypothesis environment

	1	2	3	4
Observed mean	\bar{X}_1	\bar{X}_2	\bar{X}_3	\bar{X}_4
Expected mean	μ	μ	μ	μ
Observed variance	s_1^2	s_2^2	s_3^2	s_4^2
Expected variance	σ^2	σ^2	σ^2	σ^2

- Working on sample variances:

A first estimation of σ^2 derived from samples can be found as a mathematical mean of sample variances, as simple as it is:

$$\hat{\sigma}_{s^2}^2 = \frac{s_1^2 + s_2^2 + s_3^2 + s_4^2}{4} \quad (10) \quad \boxed{10}$$

This quantity is none other than the previously discussed **MSE** (mean squares within groups) being both of them, a non-distorted valuation of the variance derived from observed variances.

- Working on sample means:

The central limit theorem assure us that data normally distributed with mean μ and variance σ^2 have the sampling distribution of means normal and characterized by mean μ and variance $\frac{\sigma^2}{n}$ (being n sample size): in this manner ($\bar{X}_1, \bar{X}_2, \bar{X}_3, \bar{X}_4$) are distributed.

Showing that a second estimation of σ^2 can be computed. Treating the observed means as raw scores, calculation of their overall mean and their variance is been made and then the latter has been multiplied by n extrapolating this:

$$\hat{\sigma}_{\bar{X}}^2 = n \frac{\sum_{i=1}^4 (\bar{x}_i - \bar{x})^2}{4 - 1} \quad (11) \quad \boxed{11}$$

This quantity is another estimation of σ^2 based on means and is no less than the already-discussed MST, the represent the same quantity: mean squares between groups.

Now two quantities, from different sources, estimate σ^2 .

Following the logic of a simple ANOVA, by doing a ratio with these variances is expected a value ≈ 1 .

$$E \left(\frac{\hat{\sigma}_{\bar{X}}^2}{\hat{\sigma}_{s^2}^2} \right) \approx 1 \quad (12) \quad \boxed{12}$$

It is simple to generalize for m groups (substituting m to 4) and easy is to recognise in this ratio the F statistic expected under the Null hypothesis.

Alternative hypothesis environment

Consideration must be given to the same computations but in the alternative hypothesis environment. Alternative hypothesis states that (at least) one of the four groups does not come from the same normal distribution as others one. To ease calculations it is assumed that all groups come from different normal distribution with different means $(\mu_1, \mu_2, \mu_3, \mu_4)$ but same variance σ^2 .

Because of the equality of variance in (10) the **MSE** calculated is still valid but not the **MST**. Considering that MST has to be extrapolated from the same formula, which now has different values for variables, it is still expected a similar value therefore it appears as follows:

$$E\left(\frac{\hat{\sigma}_{\bar{x}}^2}{\hat{\sigma}_{s^2}^2}\right) = n\sigma_{\mu}^2 - \hat{\sigma}_{\bar{x}}^2 \quad (13) \quad \boxed{13}$$

So it has to be *expected* that an F statistics computed is as follows:

$$E(F) = \frac{n\sigma_{\mu}^2 + \hat{\sigma}_{\bar{x}}^2}{\hat{\sigma}_{s^2}^2} = \frac{n\sigma_{\mu}^2}{\hat{\sigma}_{s^2}^2} + \frac{\hat{\sigma}_{\bar{x}}^2}{\hat{\sigma}_{s^2}^2} \approx \frac{n\sigma_{\mu}^2}{\hat{\sigma}_{s^2}^2} + 1 \quad (14) \quad \boxed{14}$$

This quantity will always be greater than 1 and this is WHY the larger the *F* statistic, the more unlikely the null hypothesis is going to be accepted (in other words, the more likely is false).

Approaching MANOVA All the quantities and concepts just expressed have to be adapted to vectors, matrices and to a multivariate environment therefore the following example has three dependent variables.

Null hypothesis assumes here that values on the three variables for each of the four groups have been sampled from a trivariate normal distribution of which mean vector is:

$$\mu = \begin{pmatrix} \mu_1 \\ \mu_2 \\ \mu_3 \end{pmatrix}$$

And covariance matrix is

$$\Sigma = \begin{pmatrix} \sigma_1^2 & \rho_{12}\sigma_1\sigma_2 & \rho_{13}\sigma_1\sigma_3 \\ \rho_{12}\sigma_1\sigma_2 & \sigma_2^2 & \rho_{23}\sigma_2\sigma_3 \\ \rho_{13}\sigma_1\sigma_3 & \rho_{23}\sigma_2\sigma_3 & \sigma_3^2 \end{pmatrix}$$

Null hypothesis environment

Again, the table for observed and expected statistics for a one-way MANOVA.

$$\begin{array}{l} \text{Observed mean} \\ \text{Expected mean} \\ \text{Observed variance} \\ \text{Expected variance} \end{array} \begin{pmatrix} 1 & 2 & 3 & 4 \\ \bar{\mathbf{X}}_1 & \bar{\mathbf{X}}_2 & \bar{\mathbf{X}}_3 & \bar{\mathbf{X}}_4 \\ \mu & \mu & \mu & \mu \\ \mathbf{S}_1 & \mathbf{S}_2 & \mathbf{S}_3 & \mathbf{S}_4 \\ \Sigma & \Sigma & \Sigma & \Sigma \end{pmatrix}$$

Resemblance is clearly shown, the only difference in the two tables is the notation, the latter one is, obviously, a matrical notation.

- **Working on sample variances:**

First estimation of Σ derived from samples can be found as a mathematical mean of sample variances, this time calculating an average observed covariance matrix:

$$\hat{\Sigma}_{\mathbf{w}} = \frac{\mathbf{S}_1 + \mathbf{S}_2 + \mathbf{S}_3 + \mathbf{S}_4}{4} \quad (15) \quad \boxed{15}$$

Still, same equations as (I10) but different notation.

Still, same significance, a not-distorted valuation of the variance derived from observed variances: a matrical MSE (mean squares within groups).

- **Working on sample means:**

As before, for the null hypothesis and *theorem of central limit* means are sampled from a trivariate normal distribution with μ vector of means and covariance matrix Σ/\mathbf{n} .

The same procedure as before, treating means as raw data their covariance matrix is calculated and then multiplied by \mathbf{n} resulting in:

$$\begin{array}{ccc} \bar{X}_{11} & \bar{X}_{12} & \bar{X}_{13} \\ \bar{X}_{21} & \bar{X}_{22} & \bar{X}_{23} \\ \bar{X}_{31} & \bar{X}_{32} & \bar{X}_{33} \\ \bar{X}_{41} & \bar{X}_{42} & \bar{X}_{44} \end{array}$$

where:

- \bar{X}_{ij} is the mean for the i -th group on the j -th variable

Calculating the sums of squares and cross products matrix ($\mathbf{SSCP}_{\bar{\mathbf{x}}}$) the estimation of Σ is:

$$\hat{\Sigma}_{\mathbf{b}} = n \frac{\mathbf{SSCP}_{\bar{\mathbf{x}}}}{4 - 1} \quad (16) \quad \boxed{16}$$

Still, same equations as (I11) but different notation.

Still, same significance, another estimation of the variance based on means: a matrical MST (mean squares between groups).

Again two different estimates of Σ have been computed from different sources.

Following the logic of an ANOVA production of ratio is compulsory with these covariance-variance matrices, under the null hypothesis is expected a value ≈ 1 , more precisely is expected an Identity matrix:

$$E\left(\hat{\Sigma}_{\mathbf{b}} \hat{\Sigma}_{\mathbf{w}}^{-1}\right) = \mathbf{I} \quad (17) \quad \boxed{17}$$

Identical to (I12) but in matrix notation.

Alternative hypothesis environment

Once more is assumed that each group has been sampled from a multivariate normal distribution with equal covariance matrix Σ . In this way (I15) is still a correct estimate of Σ but because for the alternative hypothesis at least one group has been sampled from a normal distribution with different mean vector the estimates of variance matrix from means has to be changed in the same way it had been done for the univariate situation previously described. The covariance matrix for the observed mean vectors $\hat{\Sigma}_{\mathbf{b}}$ will be formed, in order, by the covariance due to *true* mean differences Σ_{μ} and the covariance matrix for sampling error $\frac{\Sigma}{\mathbf{n}}$:

$$\hat{\Sigma}_{\mathbf{b}} = n\Sigma_{\mu} + \Sigma \quad (18) \quad \boxed{18}$$

Is getting clear that $\left(\frac{18}{18}\right)$ will be the same as $\left(\frac{13}{13}\right)$ but in matrix notation.

So, the “F” statistic is *expected* to be:

$$E\left(\hat{\Sigma}_b \hat{\Sigma}^{-1}\right) = (n\Sigma_\mu + \Sigma) \Sigma^{-1} = n\Sigma_\mu \Sigma + \Sigma \Sigma^{-1} = n\Sigma_\mu \Sigma + \mathbf{I} \quad (19) \quad \boxed{19}$$

As usual it has same meaning of the $\left(\frac{14}{14}\right)$. As shown before, this expectation will always be greater than 1 because, as it has been done before, the sampling error has been included in the computation.

Before approaching hypothesis testing in MANOVA the last “translation” into the “multivariate lexicon” left is the F value, being:

H the hypothesis sums of squares and cross products matrix, multivariate equivalent of SST

E the error sums of squares and cross product matrix, multivariate equivalent of SSE

A is the multivariate equivalent of the F statistic, a matrix multiplied for the ratio between the respective domains of freedom DFE/DFE .

$$\mathbf{A} = \mathbf{HE}^{-1}$$

Because most of the statistical programs uses **A** as a simplified statistic.

Hypothesis testing in MANOVA

Each one of these tests work on **A** and, being really difficult calculations, some of the methods give an exact F ratio while others give an approximation of it.

with

λ_i the i -th eigenvalue of **A**

q the number of dependent variables

- *Pillai's trace*

The most powerful and robust to violation of hypothesis such as: decreasing sample sizes, unequal cell sizes and violated homoscedasticity.

$$Pillai's\ trace = trace\left[\mathbf{H}(\mathbf{H} + \mathbf{E})^{-1}\right] = \sum_{i=1}^q \frac{\lambda_i}{1 + \lambda_i} \quad (20) \quad \boxed{20}$$

- *Hotelling-Lawley's trace*

Works on differences over all the characteristic roots.

$$Hotelling - Lawley's\ trace = trace(\mathbf{A}) = trace(\mathbf{HE}^{-1}) = \sum_{i=1}^q \lambda_i \quad (21) \quad \boxed{21}$$

- *Wilk's lambda Λ*

he most commonly used, considers differences over all the characteristic roots, the smaller it is the larger is the between-groups variability. This is why, only for big samples, the quantity $1 - \Lambda$ (being biased is dangerous to use it for small samples) can be used as the proportion of variance in the dependent variables explained by the model effect.

$$Wilk's\ lambda = \Lambda = \frac{|\mathbf{E}|}{|\mathbf{H} + \mathbf{E}|} = \prod_{i=1}^q \frac{1}{1 + \lambda_i} \quad (22) \quad \boxed{22}$$

- *Roy's largest root*

An upper bound for the F statistic, highly sensitive to violated assumptions.

$$Rois's\ largest\ root = max(\lambda_i) \quad (23) \quad \boxed{23}$$

²⁰(20) and ²³(23) results are later translated into F statistic (because of the more availability of already-printed tables), by statistical packages, to test the null hypothesis. If generated F statistics differ among the four methods Pillai's trace is the reference and Roy's largest root can help being used as the lower bound estimate of the probability of F .

Assumptions in MANOVA

The assumptions required ^{manova}[34] for the MANOVA procedure are various and sufficiently complex and therefore difficult to meet.

1. *Sample size*

- Each cell must have more elements than the number of dependent variables.
- Larger samples are used, more robust the method gets to violations of assumptions.

2. *Normality*

- MANOVA is rather robust to violation of normality but 20-30 samples per cell assures better results.
- Testing univariate normality is not a guarantor of normality but it does help with it.
- Testing multivariate normality with Mahalanobis' distance will add proofs to this assumption.

3. *Outliers*

- Checking for outliers is needed because of the very sensitivity of MANOVA to it and their impact on Type I error (both, univariate or multivariate check).
- Attention has to be paid on deleting outliers, they might be an unusual combination of the variables but still a correct one.
- MANOVA, however, can tolerate few outliers but if there are too many of them they can be deleted or the variables involved can be transformed.

4. *Linearity*

- Linear relationship among all pairs of dependent variables.
- This can be detected via scatterplots and bivariate correlations .

5. *Homogeneity of regression*

- Important only if stepdown analysis is being used (variables are arranged in descending order of importance).
- Covariates have to have a homogeneous regression effect (equal effect on the dependent variables across the groups).

6. *Multicollinearity and singularity*

- Better situation is when dependent variables are only moderately correlated.
- If correlations are low running a separate ANOVA gives better results.

- If correlations are high (0.7~0.9), redundant dependent variables decrease statistical efficiency so it is advisable to remove the most correlated pairs of variables.

7. *7.Homogeneity of variance-covariance matrix (homoscedasticity)*

- Whether there are equal sample sizes for each cell MANOVA is fairly robust to this.
- It can be tested with an F test from Box's M statistics but attention to the violation of multivariate normality for large sample sizes, towards which is very sensitive.

8. *8.Homogeneity of error variances*

- Levene's test can be used to test this assumption.
- If this is not respected it should be used a more conservative critical level for determining significance for that variable in the univariate *F*-test.

So, after all this, **why using MANOVA instead of ANOVA?**

Pro...

- Tests several effects from independent variables and outcomes with just a single analysis.
- Uses convergence (operationally defined discrete variables cannot capture the conceptual variable of interest).
- More powerful test of significance than univariate tests and interpretative advantages over a series of univariate ANOVA.
- Reduced Type I error than a series of univariate ANOVA.

...and con

- Each effect evaluated for significance uses different discriminant functions (a one factor may influence a combination of variables completely different from the combination affected from the other factor or the interaction between the two factors).
- There are a lot of assumptions which are difficult to assess and meet.

4.1.5 R and ANOVA procedure

4.1.5

How does R computes and approach the ANOVA procedure?

A clarification is needed in order to understand the set of problems which the data analysis (shown later in this report) has gone and the reasons of certain choices.

If not working on mean values this study has unbalanced data; therefore it is needed to explain the different ways to calculate the Sum of Square of ANOVA: *Type I, II, III* sum of squares (*SS*). This study has two factors **Gender** and **Age** (call **G** and **A**) and so an interaction effect (call **GA**). The different types of models can be written as follows:

- $SS(A,G,AG)$: *full model*
 - $SS(A,G)$: *model without interaction*
 - $SS(G,AG)$: *model that does not count the effects of A*
- etc...

The influence of factors and interactions can be tested also examining doing *F-tests* between the possible models and understand the differences between them. Using the statistical notation of "|" = "or" and "&" = "and" the *incremental* sums of squares are defined:

$$\begin{aligned}
SS(AG|A, G) &= SS(A, G, AG) - SS(A, G) \\
SS(A|AG, AG) &= SS(A, G, AG) - SS(G, AG) \\
SS(G|A, AG) &= SS(A, G, AG) - SS(A, AG) \\
SS(A|G) &= SS(A, G) - SS(G) \\
SS(G|A) &= SS(A, G) - SS(A)
\end{aligned}$$

For example:

$SS(AG|A, G)$ is the sum of squares for interaction *after* the main effects".

$SS(A|G)$ is the sum of squares for the main effect A *after* the G effect ignoring interactions.

The differences among the various types of Sums of Squares depend ^{Ranova} [35] on the stage of modelling reduction:

- **Type I**

- $SS(A)$ only for the A(ge) factor.
- $SS(G|A)$ factor G(ender) after factor A(ge)
- $SS(AG|G, A)$ factor interaction AG after factor G(ender) and factor A(ge)

It tests, in order: the main factor A(ge), the main effect of G(ender) *after* the effect of A(ge) and at last the interaction AG *after* the main effects. Because of this sequential nature in an unbalanced set of data different results will be given depending which effect is considered the main one. This approach tests for a difference in the *weighted* marginal means therefore results do depend on sample sizes; it tests the first factor without controlling the other one ^{Zahn} [36] and this is not the usual aim of a two-way ANOVA.

- **Type II**

- $SS(A|G)$ for factor A(ge)
- $SS(G|A)$ for factor G(ender)

It tests each main effect *after* the other one without assuming *any significant interaction* and if this is tested being true this type is more powerful than Type III.

- **Type III**

- $SS(A|G, AG)$ for factor A(ge)
- $SS(G|A, AG)$ for factor G(ender)

It tests each main effect *after* the other one *and* interaction therefore is better than Type II if there is significant interaction. Normally, if there is significant interaction, it is not of interest to understand main effects and they should not be studied because of misleading results.

If data is balanced all of these three types are equivalent.

To sum up

- With unbalanced data studying one factor while controlling the others is done with Type II or III SS.
- Type II is more powerful than Type III if there is no interaction.
- If there is interaction Type II becomes less appropriate while Type III results more powerful BUT main effects remain still rare interpretable because of the interaction presence.

Chapter 5

Results

results

5.1 Introduction

Statistical tools described in chapter 4^{maths} have been applied to the tensile test results and in this chapter relevant results of the analysis are presented. The four zones described in 3.1.4^{3.1.4} have been treated as separated entities. In each of them four mechanical quantities (cited in 3.1.3^{3.1.3}) have been analysed as independent variables. Because of the reasons described in 4.1.5^{4.1.5} the analysis is divided in:

- Two-way ANOVA without interaction
- Factorial two-way ANOVA with interaction

All of this tests worked on such a subdivision of quantities and factors; for each region are considered the following quantities:

- Thickness
- Strain at break (Peak strain)
- Peak stress
- Modulus (Maximum elastic Young's modulus)

and for each data are considered the following factors:

- Gender
 - Males: m
 - Females: f
- Age
 - youngs : y From 20 y.o. to 40 y.o.
 - middle-aged: m From 40 y.o. to 60 y.o.
 - elderly: e From 60 y.o. to 80 y.o. or more

The latter subdivision of age classes and their range has been mathematically decided by the criterion:

$$range = \frac{max - min}{k} \quad (1)$$

where

- k is the number of wanted classes

- *max min* is the maximum and minimum age in the dataset

Then range of age classes is: $range = \frac{83-22}{3} \approx 20$.

The *age* factor and **Gender** factor combines as follows:

	<i>e</i>	<i>m</i>	<i>y</i>
M	Me	Mm	My
F	Fe	Fm	Fy

Note One-way ANOVAs have not been used because the two factors might interact and this will not be recognized by the test. E.g. a quantity could be represented only by males in a specific age class. Therefore, the results could be due to the gender factor (being male) instead of the age factor (belonging to that class).

5.2 Two-way ANOVA without interaction

Because some patients were represented by multiple values while others by single ones, **mean values** have been calculated for each one, in each zone, because of the heterogeneity of the quantity. This operation alters the variance of data. By doing this the variability within each subject will not be analysed and interaction between factors will not be included.

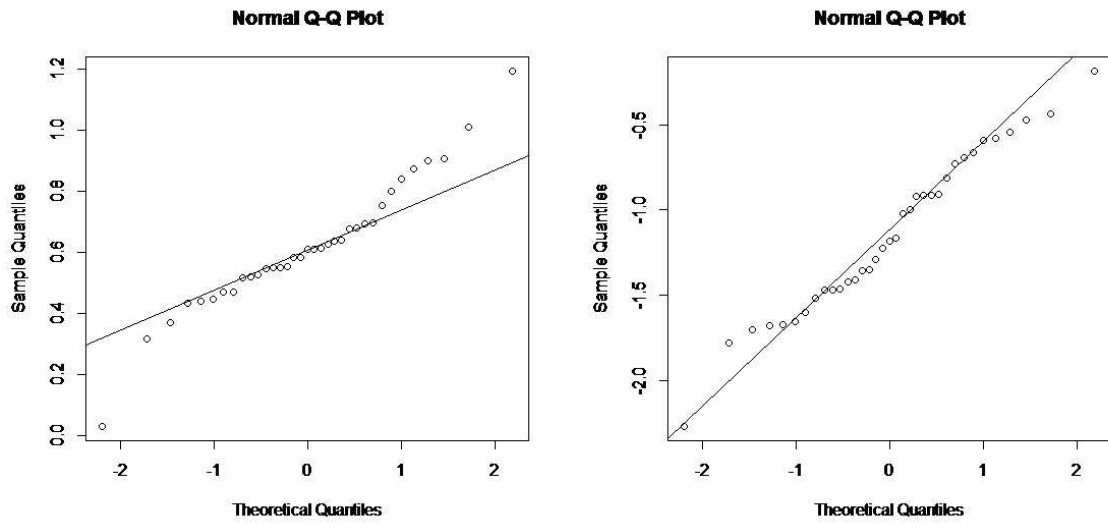
5.2.1 Visual analysis

A first impression of the plots of quantities for each zone have been plotted using mean data:

- **Q-q plots:**

In order to have informations on the data distribution, q-q plots have been graphed using mean data.

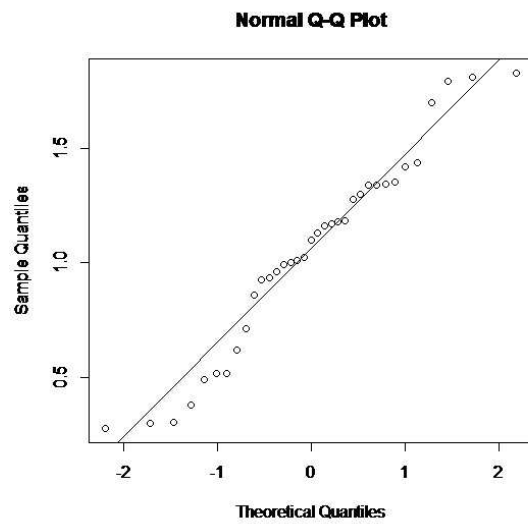
Only the following graphs show the quantities that mostly differ from a normal distribution (even if the differences still remain not too heavy) while the others (see Appendix [7.1.1](#)) did not highlight any particular deviation from normality. Shapiro-Wilk's test will later reject/accept this assertion.



(a) Thickness slightly deviates from a normal distribution on the tails. (b) Strain at break slightly deviates from a normal distribution on the tails.

qq_ac_thickres

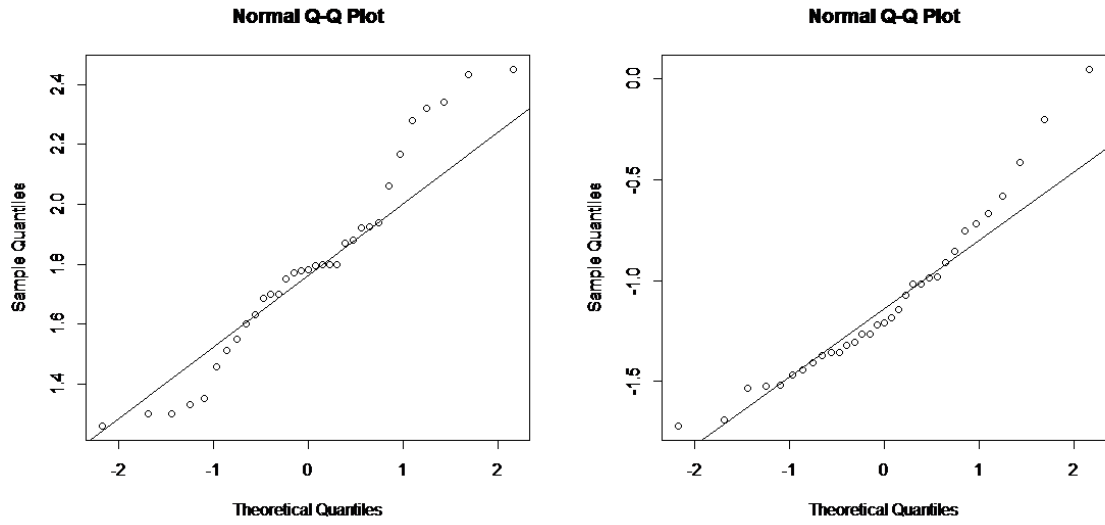
qq_ac_strainres



(c) Peak stress slightly deviates from a normal distribution on the tails.

qq_ac_peakres

Figure 5.1: q-q plots of interest of the Anterior zone Circumferential direction



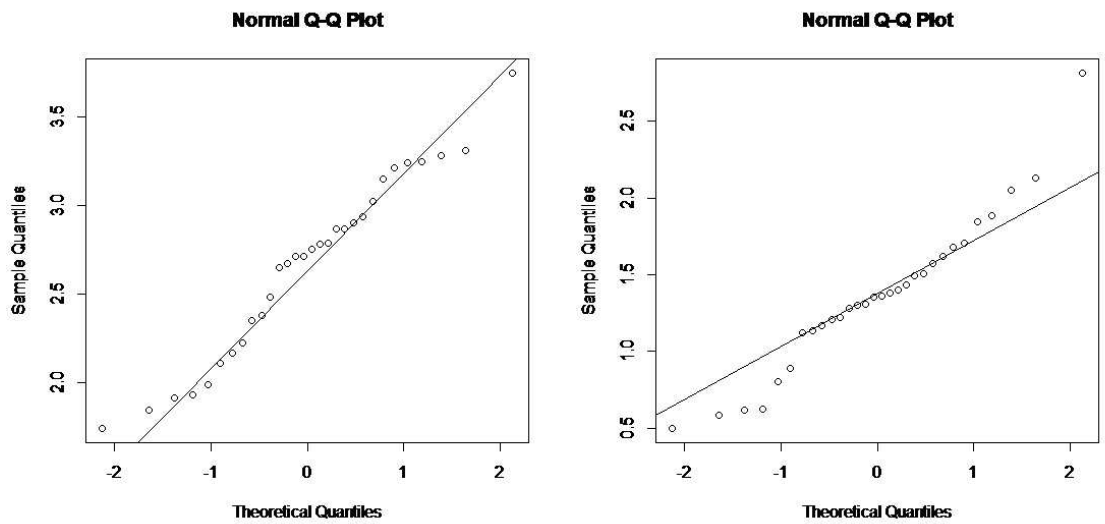
(a) Thickness slightly deviates from a normal distribution on the tails. (b) Strain at break slightly deviates from a normal distribution on the tails.

qq_al_thickres

qq_al_strainres

Figure 5.2: q-q plots of interest of the Anterior zone Longitudinal direction

– *Posterior Circumferential*



(a) Modulus slightly deviates from a normal distribution on the tails and on the centre of the distribution. (b) Only peak stress slightly deviates from a normal distribution especially on the tails of the distribution.

qq_pc_modulusres

qq_pc_peakres

Figure 5.3: q-q plots of interest of the Posterior zone Circumferential direction

– *Posterior Longitudinal*

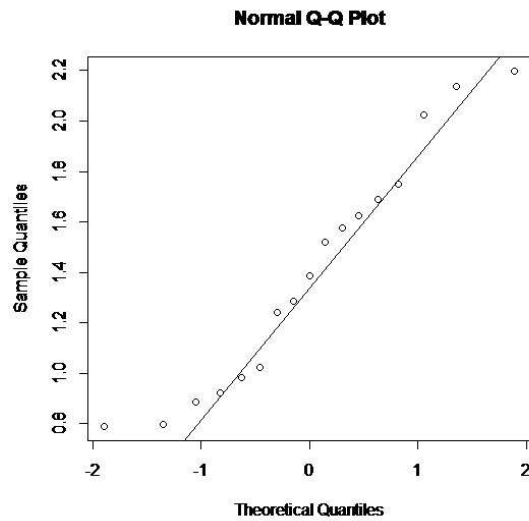


Figure 5.4: Modulus slightly deviates from a normal distribution on the tails and on the centre of the distribution, but there being very few samples, it can be considered not very significant.

qq_pl_modulusres

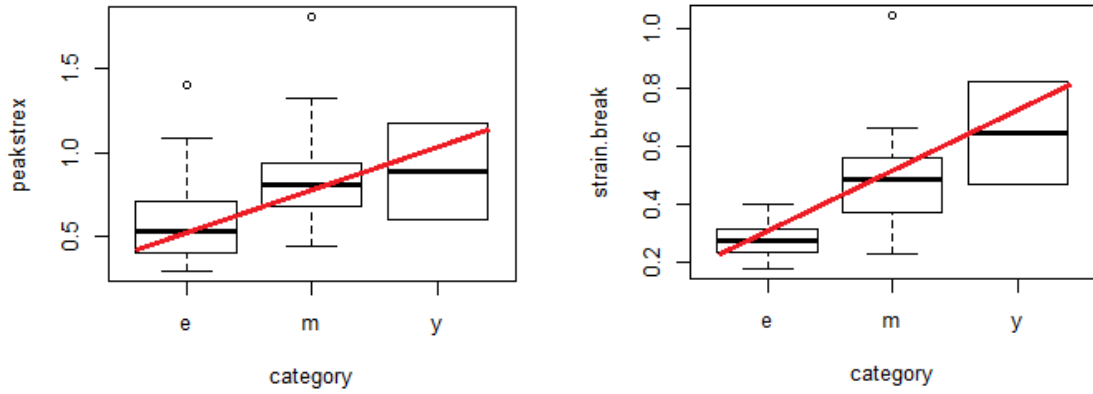
- **Box and whiskers plots:**

Each quantity of the four zones has been plotted into two box-plots, one for each factor. The aim is to have a first idea of which factor could have a visible effect, later ANOVA tests will justify/reject these effects.

Here are graphed only box-plots that showed certain trends of particular quantities in the zones while for all the others (see Appendix [7.2.1](#) for full list) no specific trends have been shown.

– *Anterior Longitudinal*

* **Age** Highlighted trend in:



(a) Peak stress among elderly middle-aged and young patients (b) Strain at break among elderly middle-aged and young patients

highboxAL_peak

highboxAL_strain

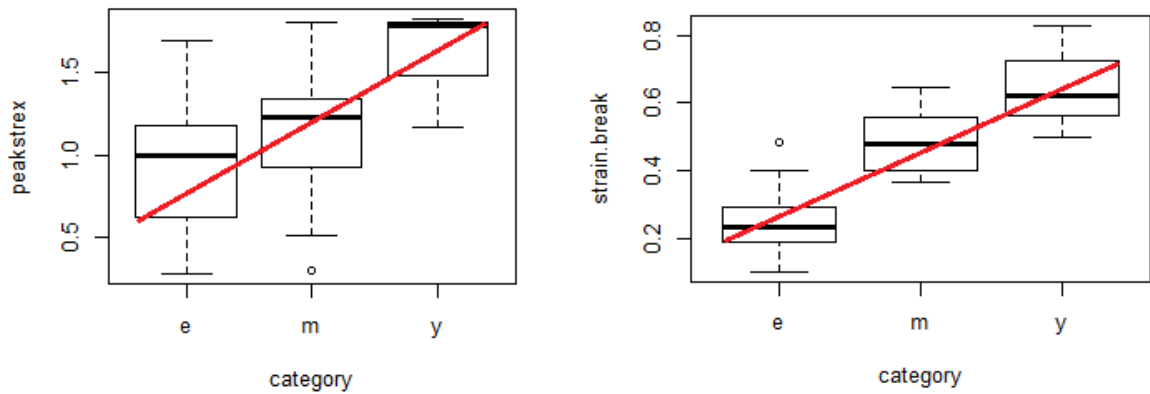
5.5

Figure 5.5: Highlighted trends in AL zone

* **Gender** No trend nor large differences were noticed.

– *Anterior Circumferential*

* **Age**
Highlighted trend in:



(a) Peak stress among elderly middle-aged and young patients (b) Strain at break among elderly middle-aged and young patients

highboxAC_peak

highboxAC_strain

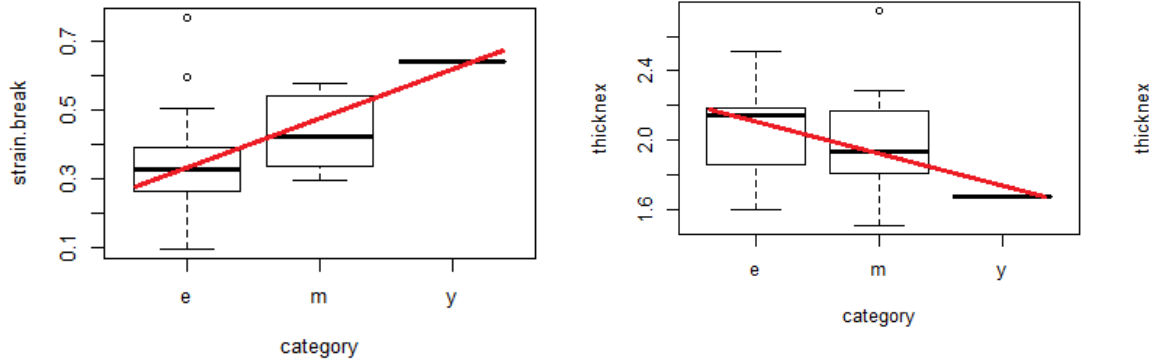
5.6

Figure 5.6: Highlighted trends in AC zone

* **Gender** No trend nor large differences were noticed.

– *Posterior Circumferential*

* **Age**
Highlighted trend in:



(a) Strain at break among elderly middle-aged and young patients
(b) Thickness among elderly middle-aged and young patients

Figure 5.7: Highlighted trends in PC zone

* **Gender** No trend nor large differences were noticed.

– *Posterior Longitudinal*

No particular trends were noticed for *Posterior Longitudinal* samples nor between gender classer nor between age classes.

After this graphical and empiric analysis it has been noticed that:

- all of the quantities sampled show a normal-like distribution without any great deviations from normality; with Shapiro-Wilk's test it will be given other reasons for this assumption.
- some quantities show a trend between age classes suggesting an influence of the last on them. Nothing showed regarding gender factor. ANOVA tests will provide, or not, justification/evidence to it.

5.2.2 Testing ANOVA assumptions

In order to proceed with ANOVA, the assumptions of Normality and Homoscedasticity of the data have to be tested with respectively, a Shapiro-Wilk's test and Levene's test (results at Appendix 7.1.2 and 7.1.3). The threshold level α is of 0.05 and it has been chosen a conservative approach.

Shapiro-Wilk's results Some test did not accept the null hypothesis at first but, after they have been log-transformed, all the samples accepted their null hypothesis of coming from a Normal distribution (see Appendix 7.1.2). The log-transformation is common in statistics because the logarithm function does not alter the data distributions, it helps indeed in reducing outliers influence.

Levene's test All tests accepted the null hypothesis of being homoscedastic. Except being made for the AL strain at break between age classes but, after a logarithmic transformation, it too resulted in being homoscedastic (see Appendix 7.1.3). As Z_{ij} (see section 4.1.3) the mathematical mean has been chosen because mean provides the best power for symmetric, moderate-tailed,

5.7

highboxPC_strain

highboxPC_thick

distributions such the observed ones.

With these test results and the previous graphical and empiric analysis it can be said that samples have the prerequisites to undergo ANOVA testing.

5.2.3 Two-way ANOVA without interaction between factors

Each quantity for each zone has been tested with a two-way ANOVA without including the interaction of age:gender factor. The threshold level α is of 0.05. However, it has not always been taken a conservative decision for p-values over the threshold level. Some of the null hypothesis have been refused even for p-values slightly over the 0.05 threshold, as boxplots and the other method results suggested. R gave in output ANOVA tables, the following gives an example of both significant (age) and non significant (gender) effect (for all the ANOVA tables see Appendix 7.3.1, for ANOVA output plots see Appendix 7.2.2).

```
> summary(aov.strain.break_al_agesex)
              Df Sum Sq Mean Sq F value    Pr(>F)
category      2  0.4934  0.24672   12.254 0.000139 ***
sex           1  0.0368  0.03682    1.829 0.186725
Residuals    29  0.5839  0.02013
---
```

5.8

Figure 5.8: ANOVA's results on strain at break from Anterior zone Longitudinal orientation

- *Anterior Longitudinal*

- Thickness: gender and age seem to have no statistical significant effect.
- Modulus: gender and age seem to have no statistical significant effect.
- **Peak stress**: only age seem to have statistical significant effect.
- **Strain at break**: only age seem to have statistical significant effect.

Without Interaction	
<i>Anterior Longitudinal</i>	
● Thickness	Age effect, <i>p-value</i> : 0.292 Gender effect, <i>p-value</i> : 0.761
● Modulus	Age effect, <i>p-value</i> : 0.443 Gender effect, <i>p-value</i> : 0.672
● Peak Stress	Age effect, <i>p-value</i> : 0.054 Gender effect, <i>p-value</i> : 0.521
● Strain at break	Age effect, <i>p-value</i> : ~0 Gender effect, <i>p-value</i> : 0.186

Figure 5.9: Summary table of p-values: in red p-values showing the effect, in black p-value showing no effect

5.9

- *Anterior Circumferential*

- Thickness: gender and age seem to have no statistical significant effect.
- Modulus: gender and age seem to have no statistical significant effect.
- **Peak stress**: only age seem to have statistical significant effect.
- **Strain at break**: only age seem to have statistical significant effect.

Without Interaction	
Anterior Circumferential	
● Thickness	Age effect, <i>p-value</i> : 0.994 Gender effect, <i>p-value</i> : 0.342
● Modulus	Age effect, <i>p-value</i> : 0.185 Gender effect, <i>p-value</i> : 0.150
● Peak Stress	Age effect, <i>p-value</i> : 0.039 Gender effect, <i>p-value</i> : 0.197
● Strain at break	Age effect, <i>p-value</i> : ~0 Gender effect, <i>p-value</i> : 0.448

Figure 5.10: Summary table of p-values: in red p-values showing the effect, in black p-value showing no effect

5.10

- *Posterior Longitudinal*

- Thickness: gender and age seem to have no statistical significant effect.
- Modulus: gender and age seem to have no statistical significant effect.
- Peak stress: gender and age seem to have no statistical significant effect.
- Strain at break: gender and age seem to have no statistical significant effect.

Without Interaction	
<i>Posterior Longitudinal</i>	
● Thickness	
	Age effect, <i>p-value</i> : 0.293
	Gender effect, <i>p-value</i> : 0.521
● Modulus	
	Age effect, <i>p-value</i> : 0.681
	Gender effect, <i>p-value</i> : 0.988
● Peak Stress	
	Age effect, <i>p-value</i> : 0.656
	Gender effect, <i>p-value</i> : 0.918
● Strain at break	
	Age effect, <i>p-value</i> : 0.460
	Gender effect, <i>p-value</i> : 0.771

Figure 5.11: Summary table of p-values: in red p-values showing the effect, in black p-value showing no effect

5.11

- *Posterior Circumferential*

- Thickness: gender and age seem to have no statistical significant effect.
- Modulus: gender and age seem to have no statistical significant effect.
- Peak stress: gender and age seem to have no statistical significant effect.
- Strain at break: gender and age seem to have no statistical significant effect.

Without Interaction	
<i>Posterior Circumferential</i>	
● Thickness	Age effect, <i>p-value</i> : 0.461 Gender effect, <i>p-value</i> : 0.470
● Modulus	Age effect, <i>p-value</i> : 0.724 Gender effect, <i>p-value</i> : 0.546
● Peak Stress	Age effect, <i>p-value</i> : 0.288 Gender effect, <i>p-value</i> : 0.158
● Strain at break	Age effect, <i>p-value</i> : 0.064 Gender effect, <i>p-value</i> : 0.196

Figure 5.12: Summary table of p-values: in red p-values showing the effect, in black p-value showing no effect

5.12

Summary

Box-plots with their trends, ANOVA tables with their p-value seem to statistically confirm the effect of age on the *strain at break* and *peak stress* of the **Anterior Circumferential** and **Anterior Longitudinal** aneurysmal aortic wall.

Note Strain at break of the *PC* zone have a slightly higher p-value than the chosen threshold $\alpha = 0.05$. Because the corresponding box-plot showed a significant trend we can assume (with a bigger uncertainty than the other results) that age could have an effect on this quantity even if the ANOVA threshold was exceeded.

5.3 Two-way factorial ANOVA-like testing with interaction

As already said this experiment has an unbalanced designed and the R-software ^{R2014}[27] cannot do a two-way unbalanced factorial ANOVA. Therefore, the following approach is not a classic ANOVA. The aim is to understand if the interaction factor (**age-gender**) has a significant effect or not on data. This approach will evaluate (via F-test) if the linear model will explain data better with or without the: interaction, age and gender effects. If "no", it would be assumed that the tested factor does not play a role on the quantities. If "yes", it would be assumed as an influencing factor on the studied quantities. No means are calculated this time and patients are represented by different numbers of data. Therefore, the big numerous data is being used.

Unfortunately this approach does not take into account the non-independence of data. In this way the main condition of independent samples results violated and this makes the test losing most of its reliability; nonetheless when its results correspond to the conclusions of the two-way ANOVA without interaction it will be used as support for the latter one.

Because of this violation a conservative decision has been made: refusing the null hypothesis (there is not an effect) **only and if only** the p-value is < 0.05 . Here are reported summarizing tables of the obtained results. In the example below the output table from R from the test on the Posterior Longitudinal peak stress where has been noticed effect of the interaction age-gender (see Appendix 7.4.2 for the complete list of output results from R).

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
picstrex ~ category * sex
              Df Sum of Sq      RSS      AIC F value Pr(>F)
<none>                0.77681 -96.976
category:sex  1    0.16873  0.94554 -93.275  5.4304 0.02816 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> mod1 <- aov(picstrex~category+sex, data=PL_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
picstrex ~ category + sex
              Df Sum of Sq      RSS      AIC F value Pr(>F)
<none>                0.94554 -93.275
category  1 0.0001090  0.94565 -95.272  0.0030 0.9568
sex       1 0.0040065  0.94955 -95.153  0.1102 0.7426
```

5.13

Figure 5.13: Results on peak stress from Posterior zone Longitudinal orientation

- *Anterior Circumferential*

- Thickness: Interaction effect age-gender is detected and, according to the without-interaction ANOVA, no effect of the main factors.
- Modulus: No interaction effect age-gender is detected and, according to the without-interaction ANOVA, there is no effect of the age factor whereas the gender factor gave opposite result.
- Peak stress: No interaction age-gender effect is detected and, according to the without-interaction ANOVA, there is effect of the age factor whereas the gender factor gave opposite result.
- Strain at break: No interaction effect age-gender is detected and, according to the without-interaction ANOVA, there is effect of the age factor but not of the gender factor.

With Interaction	
Anterior Circumferential	
● Thickness	Age effect, <i>p-value</i> : 0.640 Gender effect, <i>p-value</i> : 0.817 Interaction Age:Gender, <i>p-value</i> : 0.037
● Modulus	Age effect, <i>p-value</i> : 0.385 Gender effect, <i>p-value</i> : 0.021 Interaction Age:Gender, <i>p-value</i> : 0.398
● Peak Stress	Age effect, <i>p-value</i> : 0.013 Gender effect, <i>p-value</i> : 0.019 Interaction Age:Gender, <i>p-value</i> : 0.629
● Strain at break	Age effect, <i>p-value</i> : ~0 Gender effect, <i>p-value</i> : 0.466 Interaction Age:Gender, <i>p-value</i> : 0.799

Figure 5.14: Summary table of p-values: in red p-values showing the effect, in black p-value showing no effect

5.14

- *Anterior Longitudinal*

- Thickness: No interaction effect age-gender is detected and, contrary to the without-interaction ANOVA, is shown an effect of the age factor.
- Modulus: No interaction and main factors effects have been found, it matches with the results of the without-interaction ANOVA.
- Peak stress: No interaction effect age-gender is detected and, according to the without-interaction ANOVA, there is effect of the age factor but not of the gender factor.
- Strain at break: No interaction effect age-gender is detected and, according to the without-interaction ANOVA, there is effect of the age factor but not of the gender factor.

With Interaction	
Anterior Longitudinal	
● Thickness	<p>Age effect, <i>p-value</i>: 0.0313 Gender effect, <i>p-value</i>: 0.7582 Interaction Age:Gender, <i>p-value</i>: 0.5662</p>
● Modulus	<p>Age effect, <i>p-value</i>: 0.311 Gender effect, <i>p-value</i>: 0.724 Interaction Age:Gender, <i>p-value</i>: 0.682</p>
● Peak Stress	<p>Age effect, <i>p-value</i>: ~0 Gender effect, <i>p-value</i>: 0.449 Interaction Age:Gender, <i>p-value</i>: 0.829</p>
● Strain at break	<p>Age effect, <i>p-value</i>: ~0 Gender effect, <i>p-value</i>: 0.166 Interaction Age:Gender, <i>p-value</i>: 0.076</p>

Figure 5.15: Summary table of p-values: in red p-values showing the effect, in black p-value showing no effect

5.15

- *Posterior Circumferential*

- Thickness: No interaction and main factors effects have been found, it matches with the results of the without-interaction ANOVA.
- Modulus: No interaction effect has been found and main factors influences match with the results of the without-interaction ANOVA.
- Peak stress: Interaction effect age-gender is detected and, according to the without-interaction ANOVA, no effects of the main factors are identified.
- Strain at break: No interaction effect age-gender is detected and for the age factor a p-value of circa 0.12 supports the without-interaction ANOVA.

With Interaction	
Posterior Circumferential	
● Thickness	Age effect, <i>p-value</i> : 0.0618 Gender effect, <i>p-value</i> : 0.6479 Interaction Age:Gender, <i>p-value</i> : 0.6157
● Modulus	Age effect, <i>p-value</i> : 0.9381 Gender effect, <i>p-value</i> : 0.8305 Interaction Age:Gender, <i>p-value</i> : 0.1836
● Peak Stress	Age effect, <i>p-value</i> : 0.4691 Gender effect, <i>p-value</i> : 0.0523 Interaction Age:Gender, <i>p-value</i> : 0.0420
● Strain at break	Age effect, <i>p-value</i> : 0.1231 Gender effect, <i>p-value</i> : 0.4316 Interaction Age:Gender, <i>p-value</i> : 0.2345

Figure 5.16: Summary table of p-values: in red p-values showing the effect, in black p-value showing no effect

5.16

- *Posterior Longitudinal*

- Thickness: No interaction and main factors effects have been found, it matches with the results of the without-interaction ANOVA.
- Modulus: Interaction effect age-gender is detected and, according to the without-interaction ANOVA, no effects of the main factors are identified
- Peak stress: Interaction effect age:gender is detected and, according to the without-interaction ANOVA, no effects of the main factors are identified.
- Strain at break: No interaction and main factors effects have been found, it matches with the results of the without-interaction ANOVA.

With Interaction	
<i>Posterior Longitudinal</i>	
● Thickness	Age effect, <i>p-value</i> : 0.485 Gender effect, <i>p-value</i> : 0.878 Interaction Age:Gender, <i>p-value</i> : 0.459
● Modulus	Age effect, <i>p-value</i> : 0.976 Gender effect, <i>p-value</i> : 0.493 Interaction Age:Gender, <i>p-value</i> : 0.011
● Peak Stress	Age effect, <i>p-value</i> : 0.956 Gender effect, <i>p-value</i> : 0.742 Interaction Age:Gender, <i>p-value</i> : 0.028
● Strain at break	Age effect, <i>p-value</i> : 0.510 Gender effect, <i>p-value</i> : 0.776 Interaction Age:Gender, <i>p-value</i> : 0.082

Figure 5.17: Summary table of p-values: in red p-values showing the effect, in black p-value showing no effect

5.17

5.4 Comparisons

In the following tables results are summarized and compared; they help in understanding the informations gained from various tests. The priority given to each result is: the procedure violating the less important condition gives the most accurate results. Therefore, the final conclusions will be taken with the two-way ANOVA without interaction (which does not violate any condition), directed by box plots and supported (only and if only according) by the two-way with interaction ANOVA-like testing (which violates the independence of samples). Red p-values are the significant ones that show the presence of effects (N.B. "without interaction" wins over the "with interaction"); red arrows are for contrasting results while blue is for according results.

Without Interaction	With Interaction
<i>Anterior Circumferential</i>	
<ul style="list-style-type: none"> ● Thickness <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.994 Gender effect, <i>p-value</i>: 0.342 ● Modulus <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.185 Gender effect, <i>p-value</i>: 0.150 ● Peak Stress <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.039 Gender effect, <i>p-value</i>: 0.197 ● Strain at break <ul style="list-style-type: none"> Age effect, <i>p-value</i>: ~0 Gender effect, <i>p-value</i>: 0.448 	<ul style="list-style-type: none"> ● Thickness <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.640 Gender effect, <i>p-value</i>: 0.817 Interaction Age:Gender, <i>p-value</i>: 0.037 ● Modulus <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.385 Gender effect, <i>p-value</i>: 0.021 Interaction Age:Gender, <i>p-value</i>: 0.398 ● Peak Stress <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.013 Gender effect, <i>p-value</i>: 0.019 Interaction Age:Gender, <i>p-value</i>: 0.629 ● Strain at break <ul style="list-style-type: none"> Age effect, <i>p-value</i>: ~0 Gender effect, <i>p-value</i>: 0.466 Interaction Age:Gender, <i>p-value</i>: 0.799

6.1

Figure 5.18: Anterior zone Circumferential orientation

Without Interaction	With Interaction
<i>Anterior Longitudinal</i>	
<ul style="list-style-type: none"> ● Thickness <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.292 Gender effect, <i>p-value</i>: 0.761 ● Modulus <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.443 Gender effect, <i>p-value</i>: 0.672 ● Peak Stress <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.054 Gender effect, <i>p-value</i>: 0.521 ● Strain at break <ul style="list-style-type: none"> Age effect, <i>p-value</i>: ~0 Gender effect, <i>p-value</i>: 0.186 	<ul style="list-style-type: none"> ● Thickness <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.0313 Gender effect, <i>p-value</i>: 0.7582 Interaction Age:Gender, <i>p-value</i>: 0.5662 ● Modulus <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.311 Gender effect, <i>p-value</i>: 0.724 Interaction Age:Gender, <i>p-value</i>: 0.682 ● Peak Stress <ul style="list-style-type: none"> Age effect, <i>p-value</i>: ~0 Gender effect, <i>p-value</i>: 0.449 Interaction Age:Gender, <i>p-value</i>: 0.829 ● Strain at break <ul style="list-style-type: none"> Age effect, <i>p-value</i>: ~0 Gender effect, <i>p-value</i>: 0.166 Interaction Age:Gender, <i>p-value</i>: 0.076

Figure 5.19: Anterior zone Longitudinal orientation

Without Interaction	With Interaction
Posterior Circumferential	
<ul style="list-style-type: none"> ● Thickness <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.461 Gender effect, <i>p-value</i>: 0.470 ● Modulus <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.724 Gender effect, <i>p-value</i>: 0.546 ● Peak Stress <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.288 Gender effect, <i>p-value</i>: 0.158 ● Strain at break <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.064 Gender effect, <i>p-value</i>: 0.196 	<ul style="list-style-type: none"> ● Thickness <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.0618 Gender effect, <i>p-value</i>: 0.6479 Interaction Age:Gender, <i>p-value</i>: 0.6157 ● Modulus <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.9381 Gender effect, <i>p-value</i>: 0.8305 Interaction Age:Gender, <i>p-value</i>: 0.1836 ● Peak Stress <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.4691 Gender effect, <i>p-value</i>: 0.0523 Interaction Age:Gender, <i>p-value</i>: 0.0420 ● Strain at break <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.1231 Gender effect, <i>p-value</i>: 0.4316 Interaction Age:Gender, <i>p-value</i>: 0.2345

6.3

Figure 5.20: Posterior zone Circumferential orientation

Without Interaction	With Interaction
Posterior Longitudinal	
<ul style="list-style-type: none"> ● Thickness <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.293 Gender effect, <i>p-value</i>: 0.521 ● Modulus <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.681 Gender effect, <i>p-value</i>: 0.988 ● Peak Stress <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.656 Gender effect, <i>p-value</i>: 0.918 ● Strain at break <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.460 Gender effect, <i>p-value</i>: 0.771 	<ul style="list-style-type: none"> ● Thickness <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.485 Gender effect, <i>p-value</i>: 0.878 Interaction Age:Gender, <i>p-value</i>: 0.459 ● Modulus <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.976 Gender effect, <i>p-value</i>: 0.493 Interaction Age:Gender, <i>p-value</i>: 0.011 ● Peak Stress <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.956 Gender effect, <i>p-value</i>: 0.742 Interaction Age:Gender, <i>p-value</i>: 0.028 ● Strain at break <ul style="list-style-type: none"> Age effect, <i>p-value</i>: 0.510 Gender effect, <i>p-value</i>: 0.776 Interaction Age:Gender, <i>p-value</i>: 0.082

6.4

Figure 5.21: Posterior zone Longitudinal orientation

Observation The analysed quantities are correlated (see ^{3.1.3}3.1.3) so a MANOVA procedure, if responding to the assumptions (see ^{4.1.4}4.1.4), would be an enhanced tool of analysis. In this study, being the experiment unbalanced and not previously designed, the ratio effort-results was not worth the possible outcomes. A suggestion for a future study would be designing the uniaxial tension tests in order to operate with MANOVA instead of many ANOVAs.

Chapter 6

Conclusions

Ascending Aortic Aneurysm, AsAA, currently remains one of the most difficult cardiovascular disease to diagnose; especially their dangerous degeneration like dissection or rupture are often clinically mispredicted. Through statistical analysis of mechanical quantities of aneurysmal tissue, this study had enhanced the knowledge on factors (age and gender of the patient) that could have had an impact on such complications. Thanks to collaboration with the IRCSS Policlinico San Matteo of Pavia 46 samples of ascending aortic aneurysm, coming from just as many patients, have undergone experimental testing. Mechanical data from samples of aneurysmal ascending aortic wall have been elaborated in order to understand the effects of gender and age on their mechanical properties. These have been obtained via uniaxial tensile tests that have been performed on various specimens obtained from each one of the four subdivision (zone and direction of cut) of samples (Anterior Longitudinal, Anterior Circumferential, Posterior Longitudinal, Posterior Circumferential). The studied characteristics are: thickness of the wall, maximum achievable stiffness of the tissue (maximum Young's modulus E_{MAX}), maximal sustainable force (peak stress before rupture σ_U) and maximum sustainable deformation (strain at break ε_U). Firstly graphical analysis have been performed (q-q plots and box-plots) then two two-way ANOVA procedures analysed effects of factors, respectively, with or without their interaction.

With graphical analysis and statistical tools it can be shown that, on average, the stress and strain measured on the Anterior wall of the AsAA are influenced mostly by age. These quantities (*strain* and *stress* reached before breakage) are those which appear to differ in the age classes, in both directions (Circumferential and Longitudinal) of the Anterior arterial wall. The low p-values of the *strain at break* in the Posterior Longitudinal wall (see fig. 5.20) and its plot (see fig. 5.7a) also suggest an influence of ageing.

Therefore, **age** plays a role in the change of these AsAA mechanical properties, where the **quantities decrease as age increases**, as seen in boxplots (see fig. 5.5-5.6-5.7).

Relevant effects of the **gender** factor were not shown in any of the four quantities from any zone; neither by plots nor by most of the statistical tests.

The influence of the interaction effect (**age:gender**) has been noticed by F-tests (carried out between linear models including or not the factor) only on: *thickness* of the **AC** wall of the aorta, *peak stress* of the **PC** vasal wall, *modulus* and *peak stress* of the **PL** zone of the aortic wall.

Thence this work shows that, regardless gender, rupture of the aneurysmal wall, especially on the anterior zone, is most likely to happen in elderly patients because of the shown effect of age on the maximal force sustainable (*peak stress*) and the maximum deformation before rupture (*strain at break*).

To add histological speculations to these results, it can be said that the mean collagen proportions usually decrease in aortic aneurysms due to collagenolysis (a loss of collagen components) [37]. Furthermore, Dobrin [38] shows how elastolysis (a loss of elastin components) leads to aneurysmal-like dilation. These losses in the aneurysmal tissue could directly interfere with re-

spectively, stress and strain. Whereas researches [Hosodaetal1984](#) [39] shows no significant age-dependent changes of collagen and elastin in healthy aorta arteries. Because of the probable connection between the studied mechanical quantities and the aneurysmal condition of collagen and elastin, this work could also have suggested that age-dependent changes of the above-mentioned proteins happen in AsAA, if histological analysis would have been performed too.

This shows how much a future development with histological information is needed in order to better understand the connection between mechanical and histological aneurysmal properties. Age and gender are a priori known factors that do not need invasive measurements, likewise are genetic information or co-morbidities presence. All those factors that can be verified without heavily invasive procedures will help in unifying the mechanical properties and the histo-biological characteristics. The latter can easily be inspected non-invasively and this will help in reaching the ambitious, final goal of a minimal invasive diagnosis of AsAA via, for example, medical imaging or genetic studies.

Chapter 7

Appendix

graphs

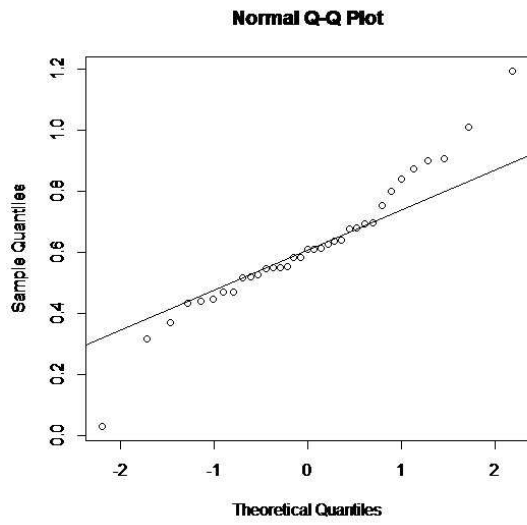
Here are collected all the graphs and tables used in the statistical analysis.

7.1 Preliminary graphs and tables from test on ANOVA assumptions on mean data

7.1.1 Quantile-Quantile Plots of the averaged data

7.1.1

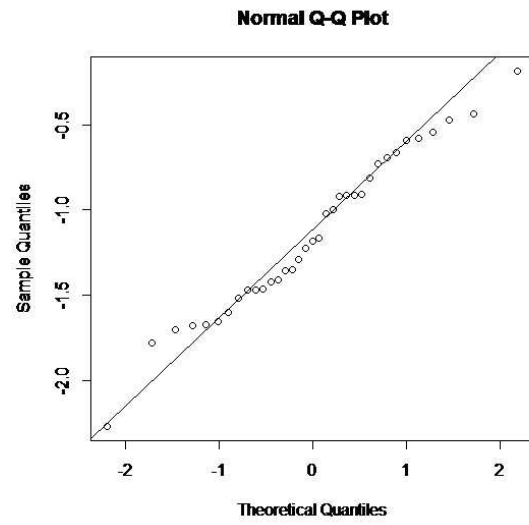
- *Anterior Circumferential*



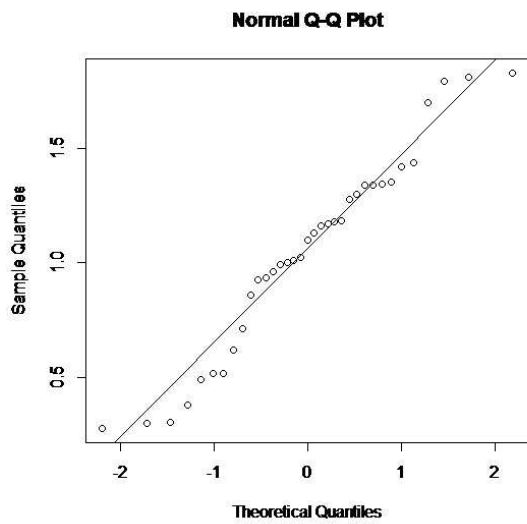
qq_ac_thick

(a) Thickness

qq_ac_strain



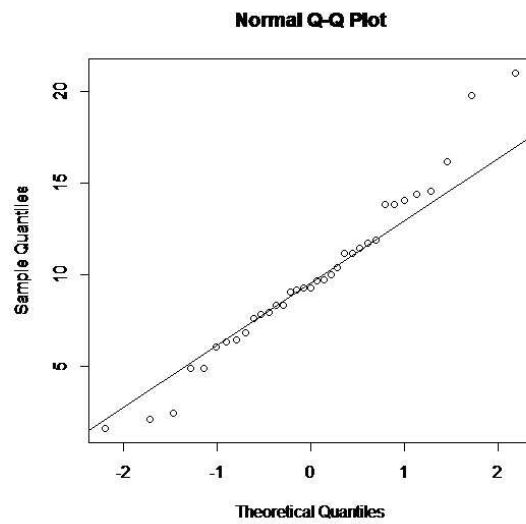
(b) Strain at break



qq_ac_peak

(c) Peak stress

qq_ac_modulus

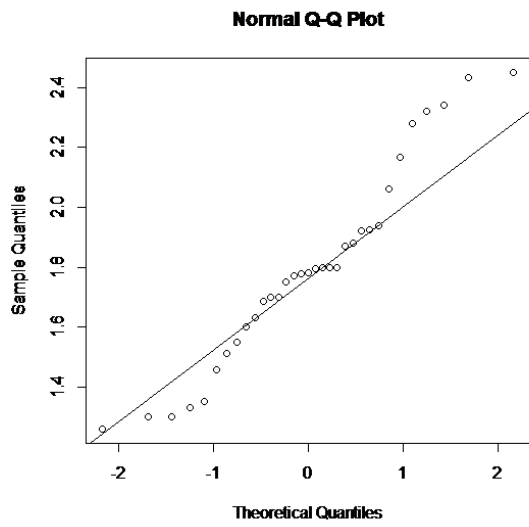


(d) Modulus

qq_ac

Figure 7.1: QQplots of AC quantities

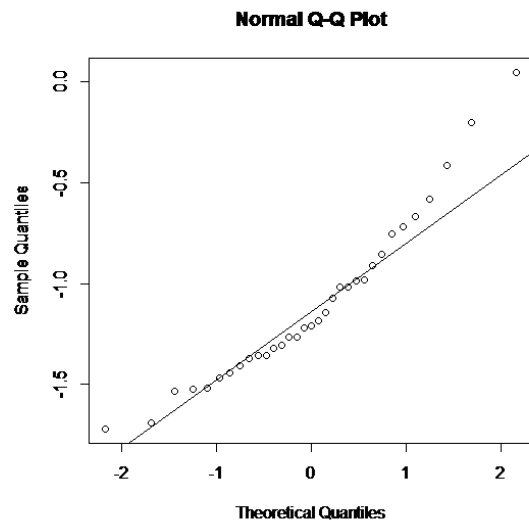
• *Anterior Longitudinal*



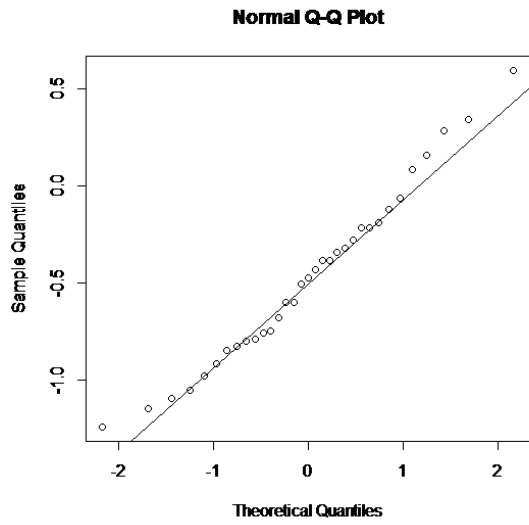
qq_al_thick

(a) Thickness

qq_al_strain



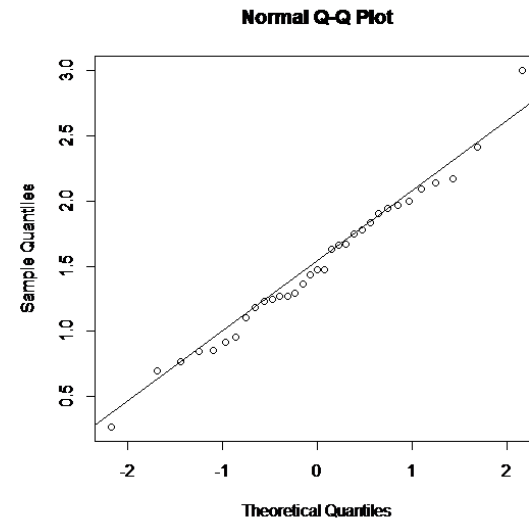
(b) Strain at break



qq_al_peak

(c) Peak stress

qq_al_modulus

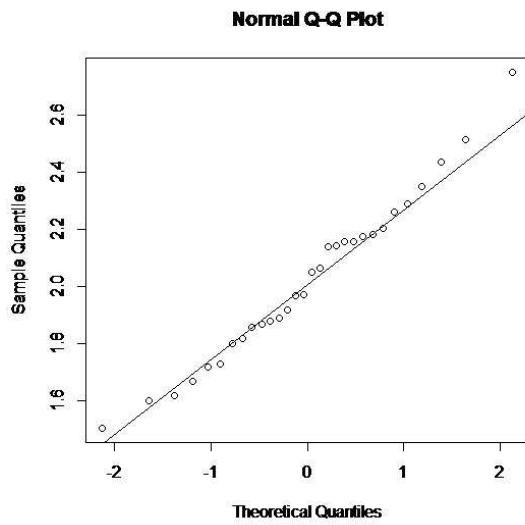


(d) Modulus

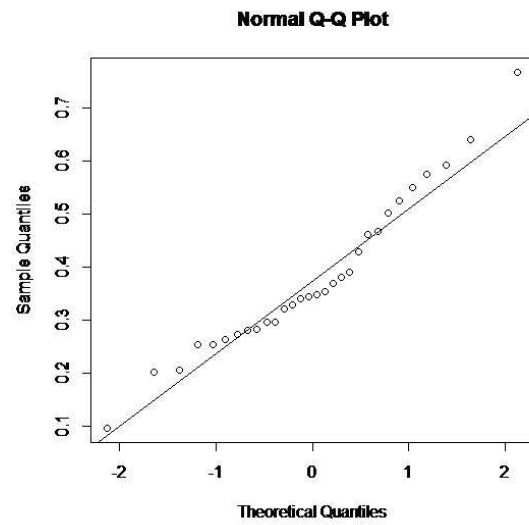
qq_al

Figure 7.2: QQplots of AL quantities

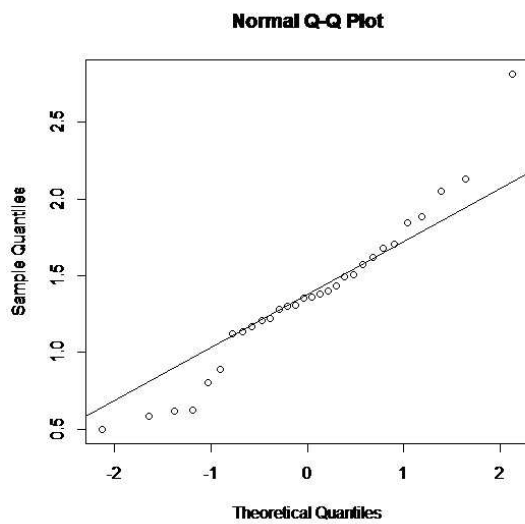
- *Posterior Circumferential*



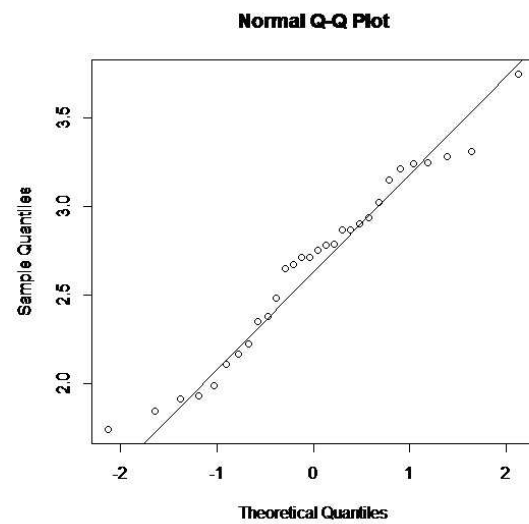
(a) Thickness

`qq_pc_thick``qq_pc_strain`

(b) Strain at break



(c) Peak stress

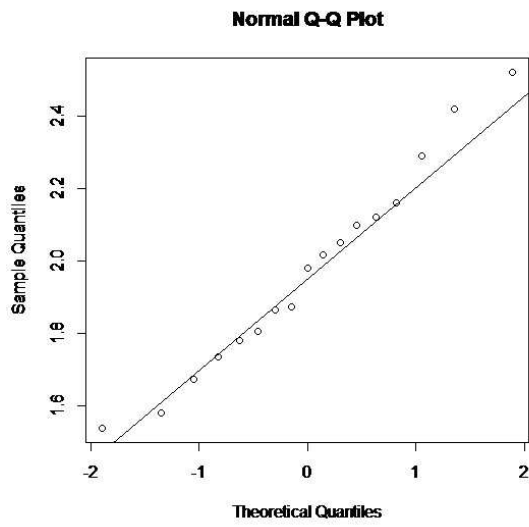
`qq_pc_peak``qq_pc_modulus`

(d) Modulus

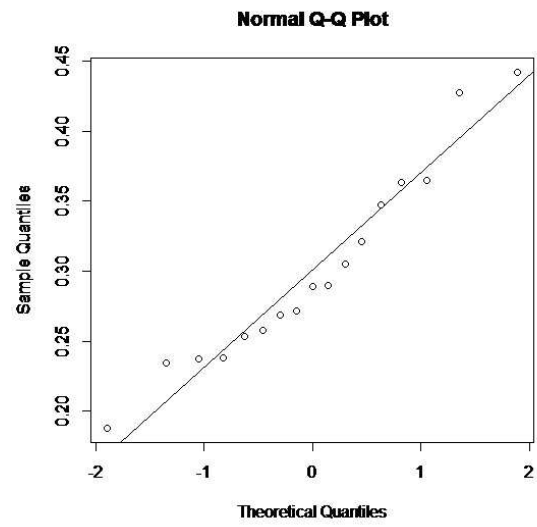
`qq_pc`

Figure 7.3: QQplots of PC quantities

• *Posterior Longitudinal*



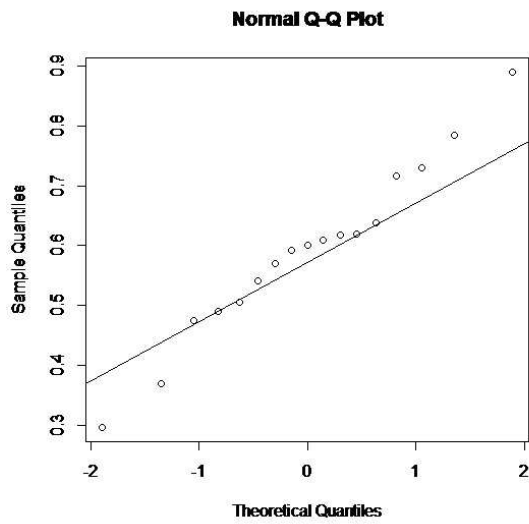
(a) Thickness



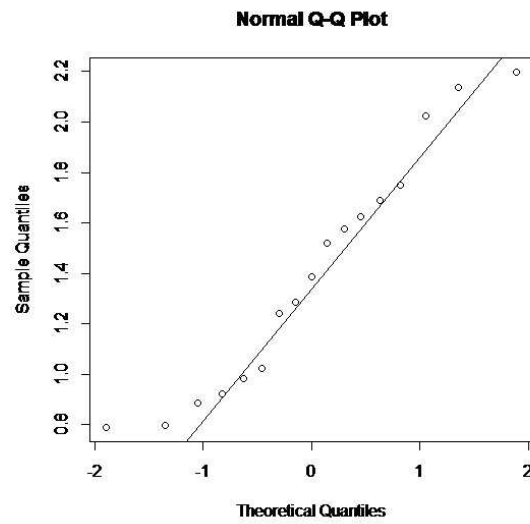
(b) Strain at break

qq_pl_thick

qq_pl_strain



(c) Peak stress



(d) Modulus

qq_pl_peak

qq_pl_modulus

qq_pl

Figure 7.4: QQplots of PL quantities

7.1.2 Shapiro-Wilks' tests results


7.1.2

- *Anterior Circumferential*

```
> shapiro.test(mean_AC[,2])

      Shapiro-Wilk normality test
```


```
data: mean_AC[, 2]
W = 0.9138, p-value = 0.009477
```

thickness 

```
> shapiro.test(mean_AC[,3])

      Shapiro-Wilk normality test
```


```
data: mean_AC[, 3]
W = 0.926, p-value = 0.02138
```

strain at break 

```
> shapiro.test(mean_AC[,4])

      Shapiro-Wilk normality test
```


```
data: mean_AC[, 4]
W = 0.9577, p-value = 0.1942
```

peak stress 

```
> shapiro.test(mean_AC[,5])

      Shapiro-Wilk normality test
```

```
data: mean_AC[, 5]
W = 0.9718, p-value = 0.4939
```

modulus 


AC_shap

(a) Shapiro's results

```
> shapiro.test(log(mean_AC[,2]))

      Shapiro-Wilk normality test
```


```
data: log(mean_AC[, 2])
W = 0.9594, p-value = 0.2199
```

thickness 

```
> shapiro.test(log(mean_AC[,3]))

      Shapiro-Wilk normality test
```

```
data: log(mean_AC[, 3])
W = 0.977, p-value = 0.6611
```

strain at break 

AC_shap_log

(b) Shapiro's results after a log-transformation

- *Anterior Longitudinal*

```
> shapiro.test(mean_AL[,2])
```

thickness



```
Shapiro-Wilk normality test
```

```
data: mean_AL[, 2]
W = 0.9505, p-value = 0.1377
```

```
> shapiro.test(mean_AL[,3])
```

strain at break



```
Shapiro-Wilk normality test
```

```
data: mean_AL[, 3]
W = 0.7778, p-value = 1.253e-05
```

```
> shapiro.test(mean_AL[,4])
```

peak stress



```
Shapiro-Wilk normality test
```

```
data: mean_AL[, 4]
W = 0.8733, p-value = 0.001162
```

```
> shapiro.test(mean_AL[,5])
```

modulus



```
Shapiro-Wilk normality test
```

```
data: mean_AL[, 5]
W = 0.7784, p-value = 1.282e-05
```

(a) Shapiro's results

AL_shap

```
> shapiro.test(log(mean_AL[,3]))
```

strain at break



```
Shapiro-Wilk normality test
```

```
data: log(mean_AL[, 3])
W = 0.9362, p-value = 0.05263
```

```
> shapiro.test(log(mean_AL[,4]))
```

peak stress



```
Shapiro-Wilk normality test
```

```
data: log(mean_AL[, 4])
W = 0.9778, p-value = 0.7177
```

```
> shapiro.test(log(mean_AL[,5]))
```

modulus



```
Shapiro-Wilk normality test
```

```
data: log(mean_AL[, 5])
W = 0.9873, p-value = 0.9596
```

(b) Shapiro's results after a log-transformation

AL_shap_log

- *Posterior Circumferential*

```
> shapiro.test(mean_PC[,2])

      Shapiro-Wilk normality test

data:  mean_PC[, 2]
W = 0.9813, p-value = 0.8592
```

thickness ✓

```
> shapiro.test(mean_PC[,3])

      Shapiro-Wilk normality test

data:  mean_PC[, 3]
W = 0.9575, p-value = 0.2677
```

strain at break ✓

```
> shapiro.test(mean_PC[,4])

      Shapiro-Wilk normality test

data:  mean_PC[, 4]
W = 0.9565, p-value = 0.2517
```

peak stress ✓

```
> shapiro.test(mean_PC[,5])

      Shapiro-Wilk normality test

data:  mean_PC[, 5]
W = 0.9142, p-value = 0.01906
```

modulus ✗

PC_shap

(a) Shapiro's results

```
> shapiro.test(log(mean_PC[,5]))

      Shapiro-Wilk normality test

data:  log(mean_PC[, 5])
W = 0.9665, p-value = 0.4476
```

modulus ✓

PC_shap_log

(b) Shapiro's results after a log-transformation

- *Posterior Longitudinal*

```

> shapiro.test(mean_PL[,2])
Shapiro-Wilk normality test
data: mean_PL[, 2]
W = 0.9743, p-value = 0.8887
> shapiro.test(mean_PL[,3])
Shapiro-Wilk normality test
data: mean_PL[, 3]
W = 0.9447, p-value = 0.378
> shapiro.test(mean_PL[,4])
Shapiro-Wilk normality test
data: mean_PL[, 4]
W = 0.9794, p-value = 0.9519
> shapiro.test(mean_PL[,5])
Shapiro-Wilk normality test
data: mean_PL[, 5]
W = 0.8866, p-value = 0.04068

```

thickness ✓

strain at break ✓

peak stress ✓

modulus ✗

PL_shap

(a) Shapiro's results

```

> shapiro.test(log(mean_PL[,5]))
Shapiro-Wilk normality test
data: log(mean_PL[, 5])
W = 0.9364, p-value = 0.278

```

modulus ✓

PL_shap_log

(b) Shapiro's results after a log-transformation

7.1.3

7.1.3 Levene's tests results

- *Anterior Circumferential*

```
> levene.test(thicknex, group, location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: thicknex
Test Statistic = 0.1913, p-value = 0.8265

> levene.test(strain.break, group, location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: strain.break
Test Statistic = 1.4543, p-value = 0.2446

> levene.test(peakstrex, group, location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: peakstrex
Test Statistic = 0.023, p-value = 0.9772

> levene.test(modulus, group, location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: modulus
Test Statistic = 0.7265, p-value = 0.4893
```

(a) Levene for age classes

AC_lev_age

```
> levene.test(thicknex, group, location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: thicknex
Test Statistic = 1.1588, p-value = 0.2895

> levene.test(strain.break, group, location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: strain.break
Test Statistic = 0.1113, p-value = 0.7408

> levene.test(peakstrex, group, location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: peakstrex
Test Statistic = 0.0034, p-value = 0.9541

> levene.test(modulus, group, location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: modulus
Test Statistic = 3.3058, p-value = 0.07812
```

(b) Levene for gender classes

AC_lev_sex

lev_AC

Figure 7.9: AC Levene's results

- *Anterior Longitudinal*

```
> levene.test(thicknex, group,location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )

data:  thicknex
Test Statistic = 0.6076, p-value = 0.5512

> levene.test(strain.break, group,location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )

data:  strain.break
Test Statistic = 6.2426, p-value = 0.005411

> levene.test(peakstrex, group,location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )

data:  peakstrex
Test Statistic = 0.8891, p-value = 0.4216

> levene.test(modulus, group,location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )

data:  modulus
Test Statistic = 1.5369, p-value = 0.2315
```

(a) Levene for age classes

AL_lev_age

```
> levene.test(thicknex, group,location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )

data:  thicknex
Test Statistic = 0.3446, p-value = 0.5614

> levene.test(strain.break, group,location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )

data:  strain.break
Test Statistic = 2.6779, p-value = 0.1119

> levene.test(peakstrex, group,location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )

data:  peakstrex
Test Statistic = 2e-04, p-value = 0.9891

> levene.test(modulus, group,location="mean")

      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )

data:  modulus
Test Statistic = 0.3339, p-value = 0.5675
```

(b) Levene for gender classes

AL_lev_sex

lev_AL

Figure 7.10: AL Levene's results

- *Posterior Circumferential*

```
> levene.test(thicknex, group, location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: thicknex
Test Statistic = 1.1567, p-value = 0.3296
> levene.test(strain.break, group, location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: strain.break
Test Statistic = 0.6578, p-value = 0.5261
> levene.test(peakstrex, group, location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: peakstrex
Test Statistic = 0.9227, p-value = 0.4096
> levene.test(modulus, group, location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: modulus
Test Statistic = 0.7173, p-value = 0.4971
```

(a) Levene for age classes

PC_lev_age

```
> levene.test(thicknex, group, location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: thicknex
Test Statistic = 0.6814, p-value = 0.4161
> levene.test(strain.break, group, location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: strain.break
Test Statistic = 0.0105, p-value = 0.919
> levene.test(peakstrex, group, location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: peakstrex
Test Statistic = 0.064, p-value = 0.8021
> levene.test(modulus, group, location="mean")
      classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not set to median )
data: modulus
Test Statistic = 0.7174, p-value = 0.4971
```

(b) Levene for gender classes

PC_lev_sex

lev_PC

Figure 7.11: PC Levene's results

- *Posterior Longitudinal*

```
> levene.test(thicknex, group, location="mean")

classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not
set to median )

data: thicknex
Test Statistic = 0.9023, p-value = 0.3572
> levene.test(strain.break, group, location="mean")

classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not
set to median )

data: strain.break
Test Statistic = 0.0044, p-value = 0.9481
> levene.test(peakstrex, group, location="mean")

classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not
set to median )

data: peakstrex
Test Statistic = 1.9933, p-value = 0.1784
> levene.test(modulus, group, location="mean")

classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not
set to median )

data: modulus
Test Statistic = 0.3081, p-value = 0.587
```

(a) Levene for age classes

PL_lev_age

```
> levene.test(thicknex, group, location="mean")

classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not
set to median )

data: thicknex
Test Statistic = 0.3303, p-value = 0.574
> levene.test(strainbreak, group, location="mean")

classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not
set to median )

data: strainbreak
Test Statistic = 0.1918, p-value = 0.6677
> levene.test(peakstrex, group, location="mean")

classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not
set to median )

data: peakstrex
Test Statistic = 0.0168, p-value = 0.8986
> levene.test(modulus, group, location="mean")

classical Levene's test based on the absolute deviations from the mean ( none not applied because the location is not
set to median )

data: modulus
Test Statistic = 0.0307, p-value = 0.8633
```

(b) Levene for gender classes

PL_lev_sex

lev_PL

Figure 7.12: PL Levene's results

Here is the Anterior Longitudinal strain at break for age classes tested after a log transformation

```
> levene.test(log(strain.break), group, location="mean")

classical Levene's test based on the absolute deviations from the mean
( none not applied because the location is not set to median )

data: log(strain.break)
Test Statistic = 2.7453, p-value = 0.08037
```

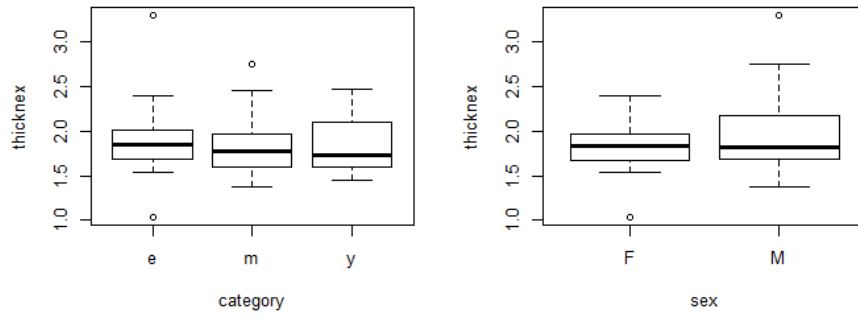
AL_lev_age_log

7.2 Box-plots and two-way ANOVA plots on mean data

7.2.1 Box-plots

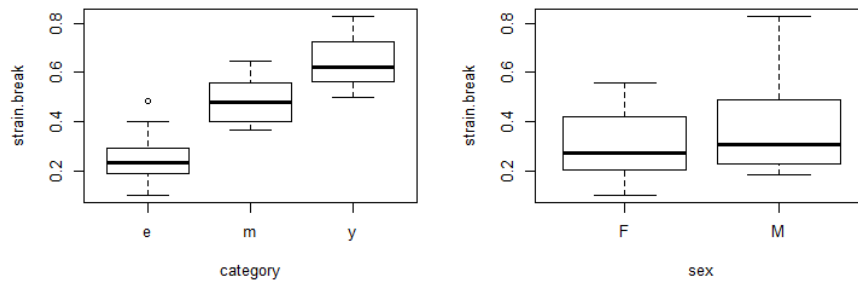
7.2.1

- *Anterior Circumferential*



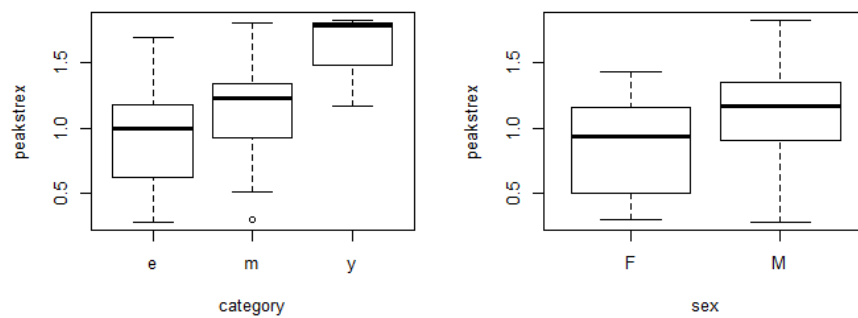
box_ac_thick

Figure 7.13: Thickness



box_ac_strain

Figure 7.14: Strain at break



box_ac_peak

Figure 7.15: Peak stress

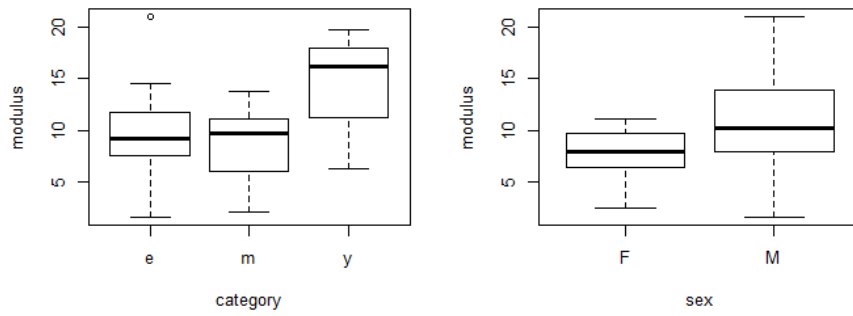


Figure 7.16: Modulus

box_ac_modulus

- *Anterior Longitudinal*

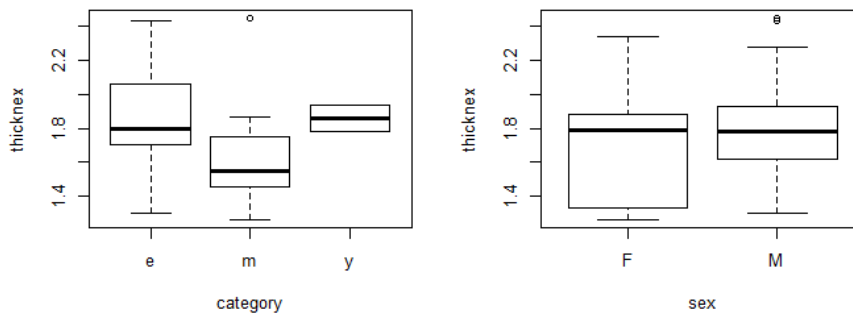


Figure 7.17: Thickness

box_al_thick

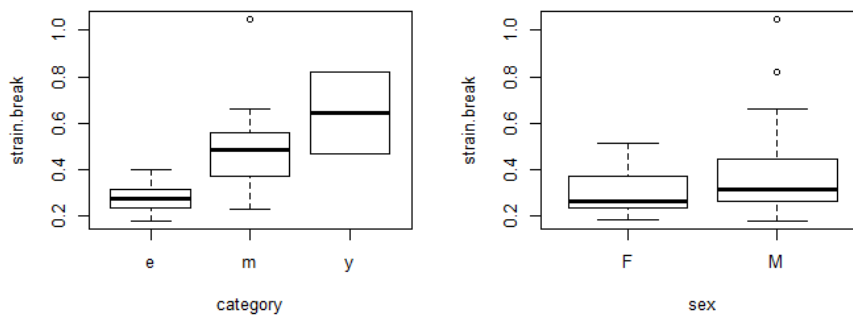


Figure 7.18: Strain at break

box_al_strain

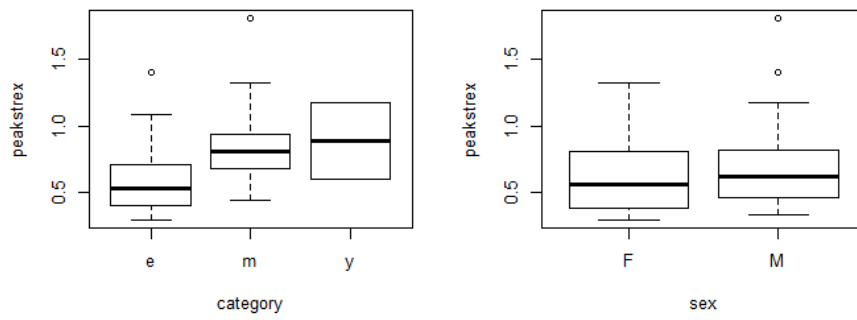


Figure 7.19: Peak stress

box_al_peak

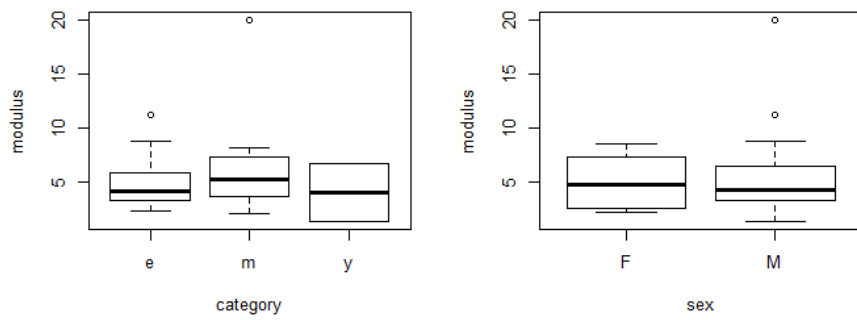


Figure 7.20: Modulus

box_al_modulus

- *Posterior Circumferential*

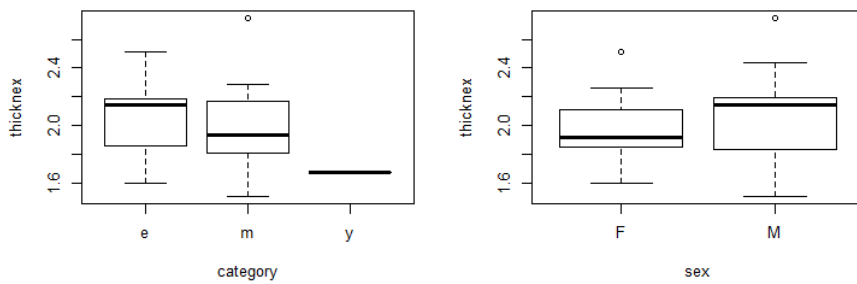
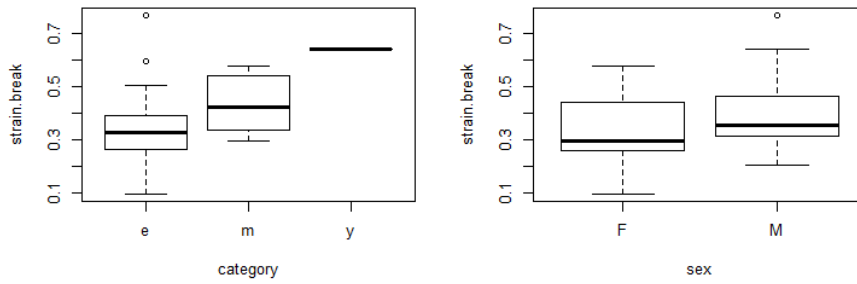


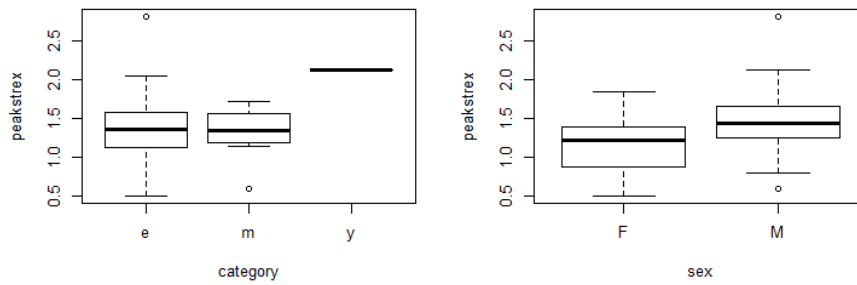
Figure 7.21: Thickness

box_pc_thick



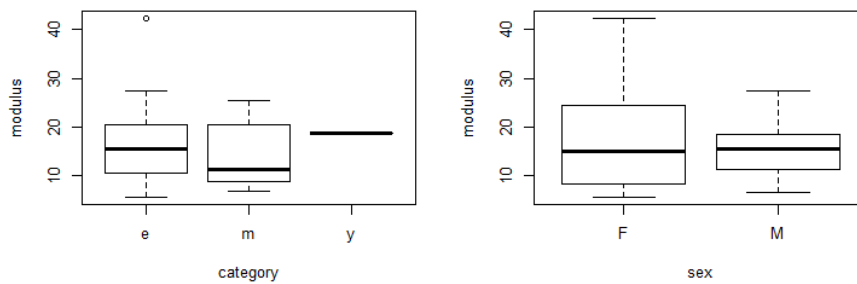
box_pc_strain

Figure 7.22: Strain at break



box_pc_peak

Figure 7.23: Peak stress



box_pc_modulus

Figure 7.24: Modulus

- *Posterior Longitudinal*

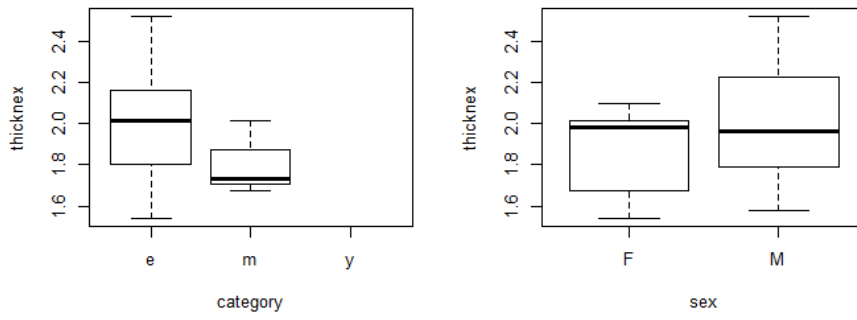


Figure 7.25: Thickness

box_pl_thick

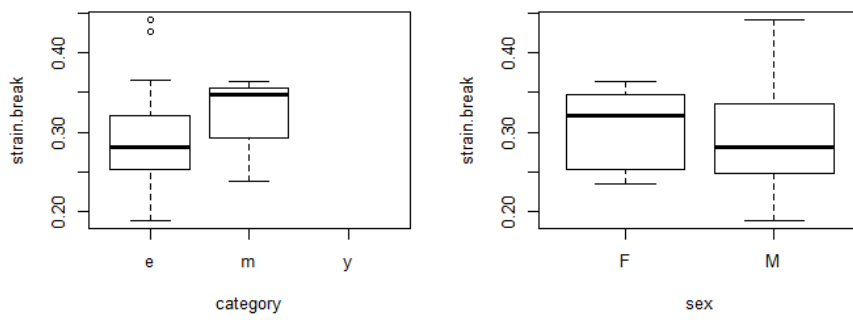


Figure 7.26: Strain at break

box_pl_strain

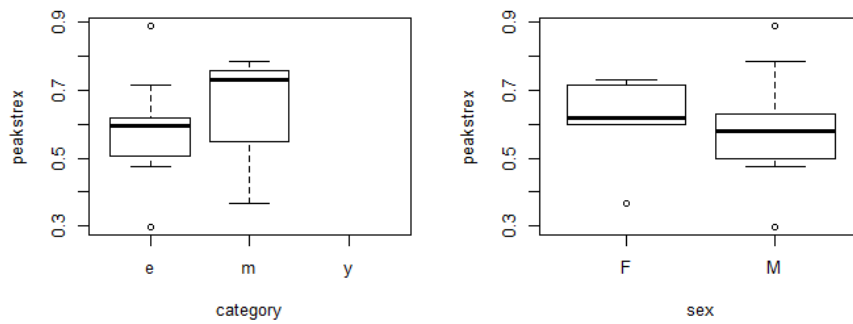


Figure 7.27: Peak stress

box_pl_peak

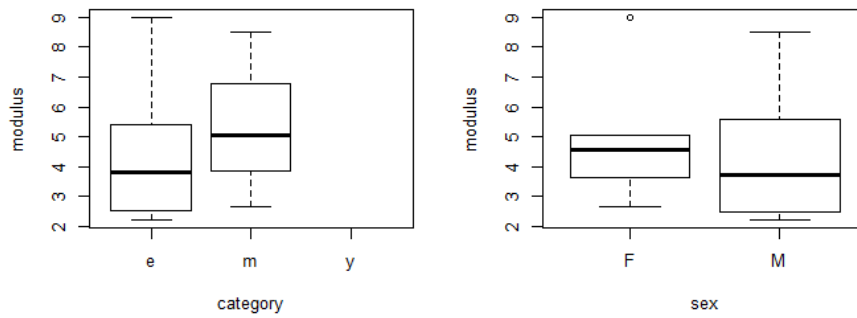


Figure 7.28: Modulus

box_pl_modulus

7.2.2 Two-way ANOVA without interaction plots

7.2.2

Anova plot output from tests on mean data.

- *Anterior Circumferential*

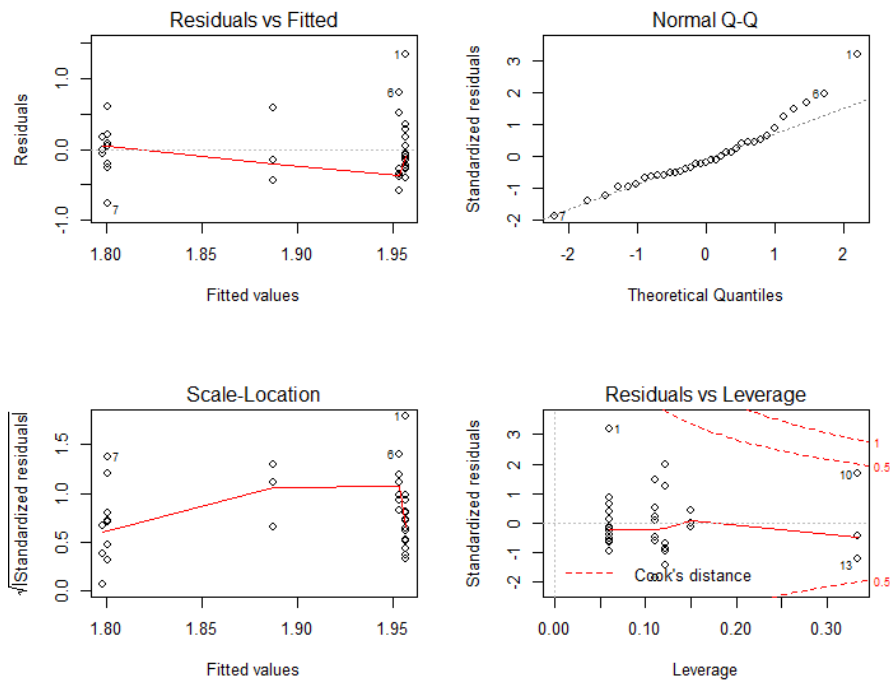


Figure 7.29: Thickness

anova_ac_thick

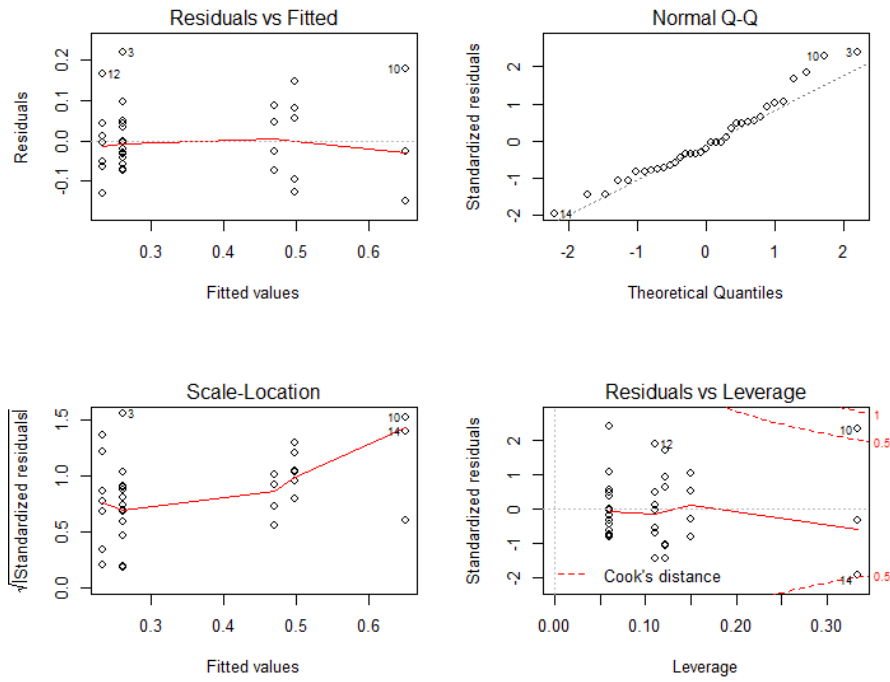


Figure 7.30: Strain at break

anova_ac_strain

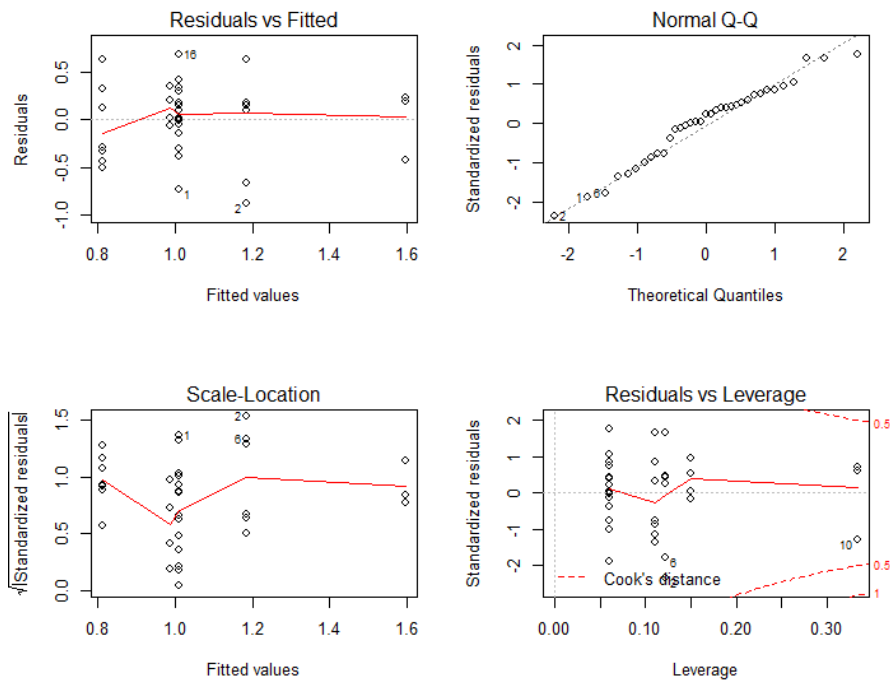


Figure 7.31: Peak stress

anova_ac_peak

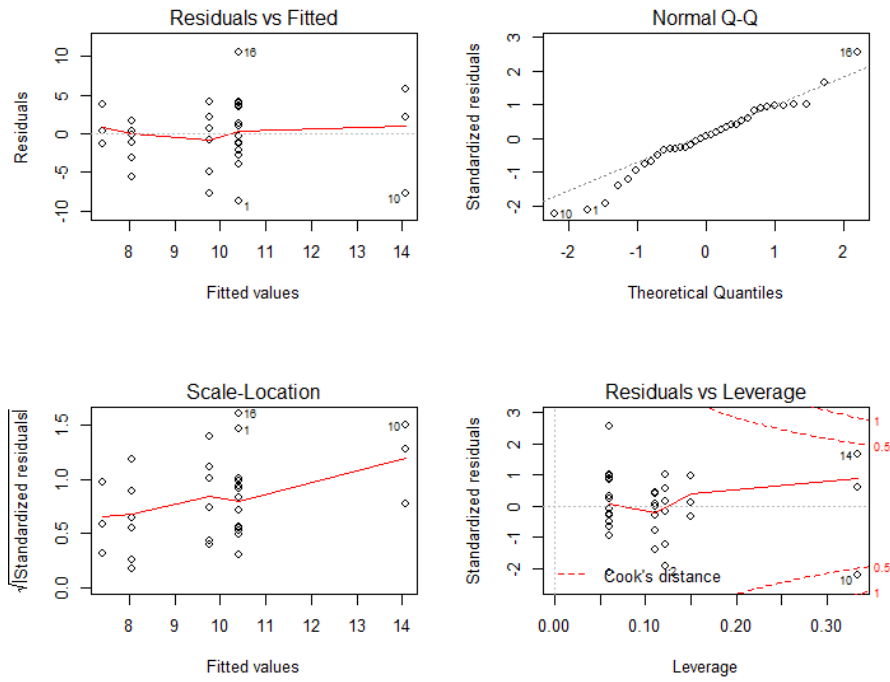


Figure 7.32: Modulus

- *Anterior Longitudinal*

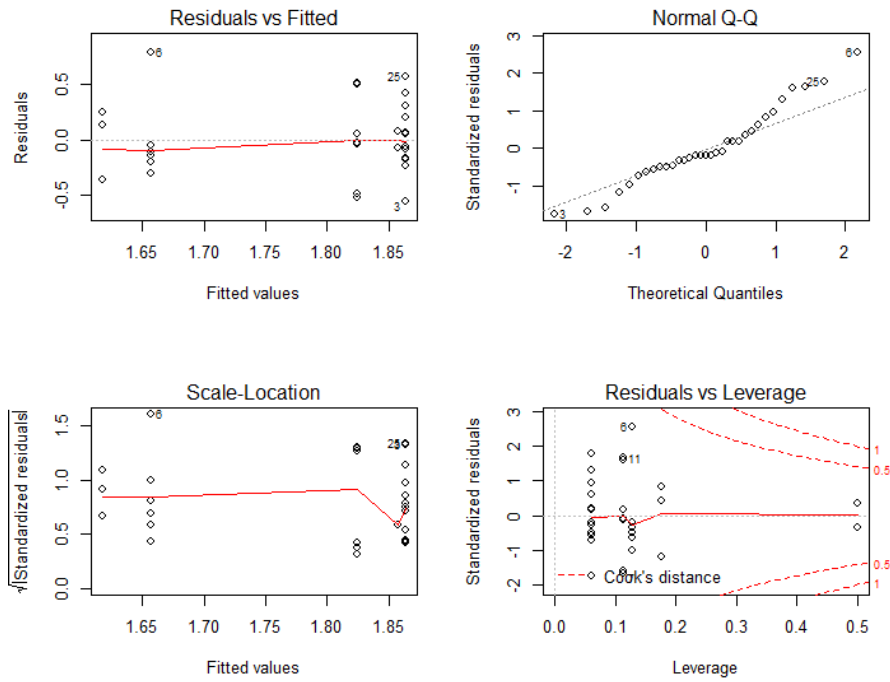


Figure 7.33: Thickness

anova_ac_modulus

anova_al_thick

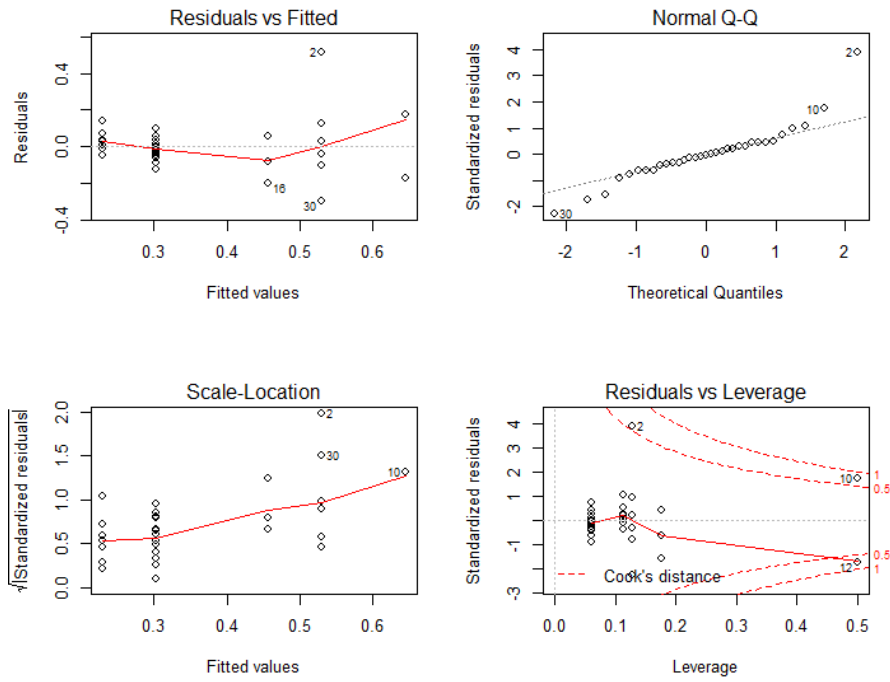


Figure 7.34: Strain at break

anova_al_strain

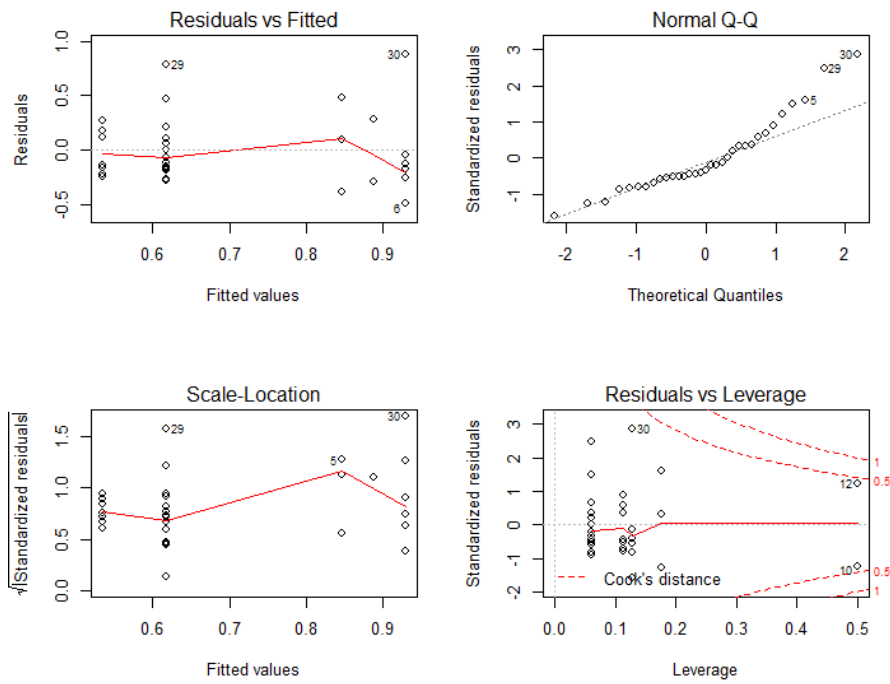


Figure 7.35: Peak stress

anova_al_peak

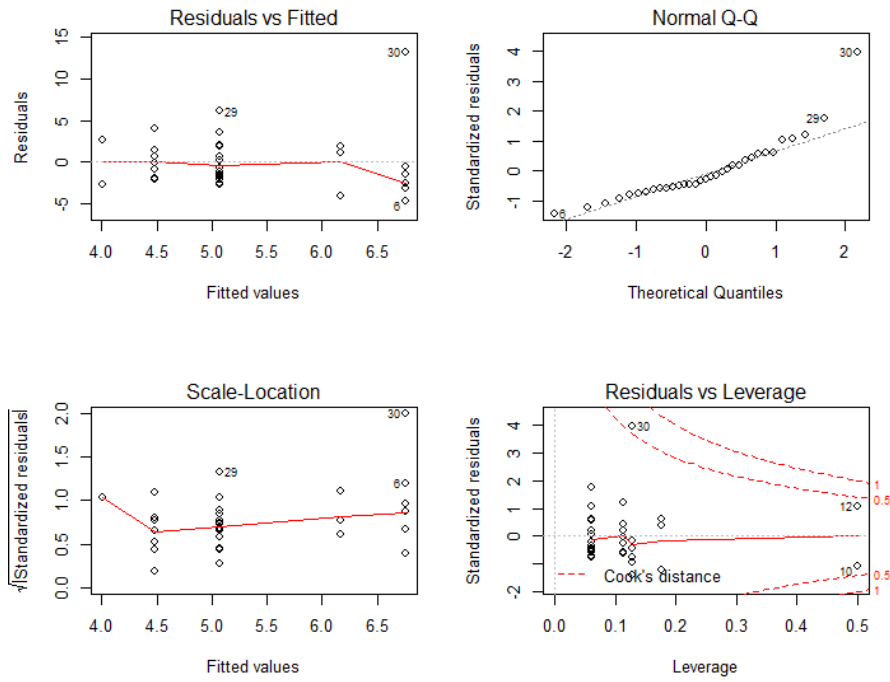


Figure 7.36: Modulus

anova_al_modulus

• *Posterior Circumferential*

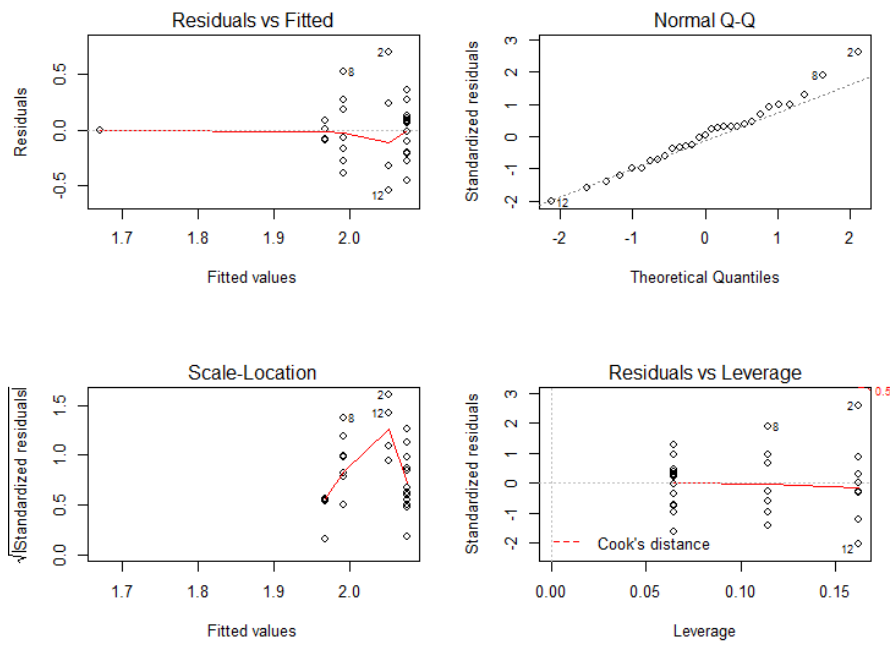


Figure 7.37: Thickness

anova_pc_thick

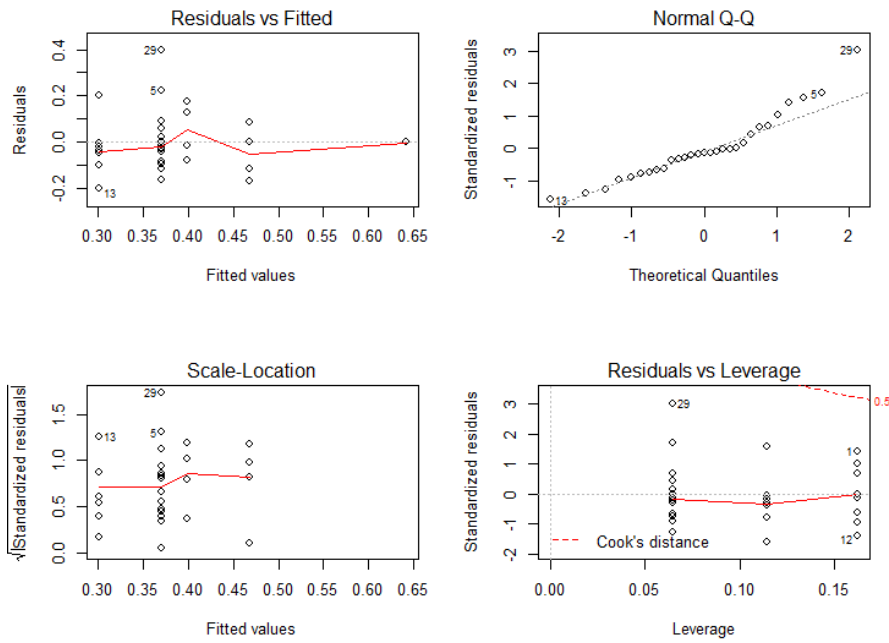


Figure 7.38: Strain at break

anova_pc_strain

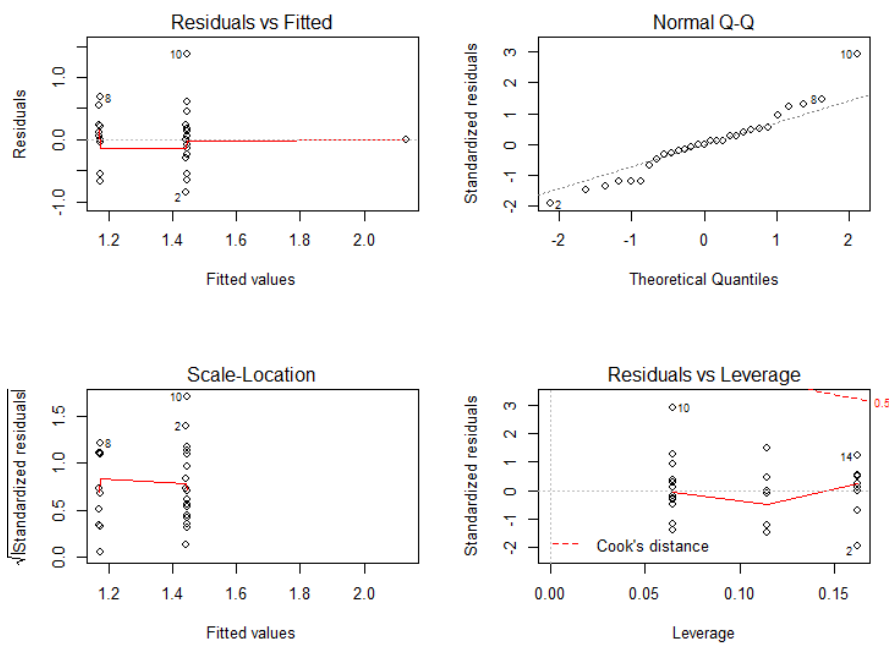


Figure 7.39: Peak stress

anova_pc_peak

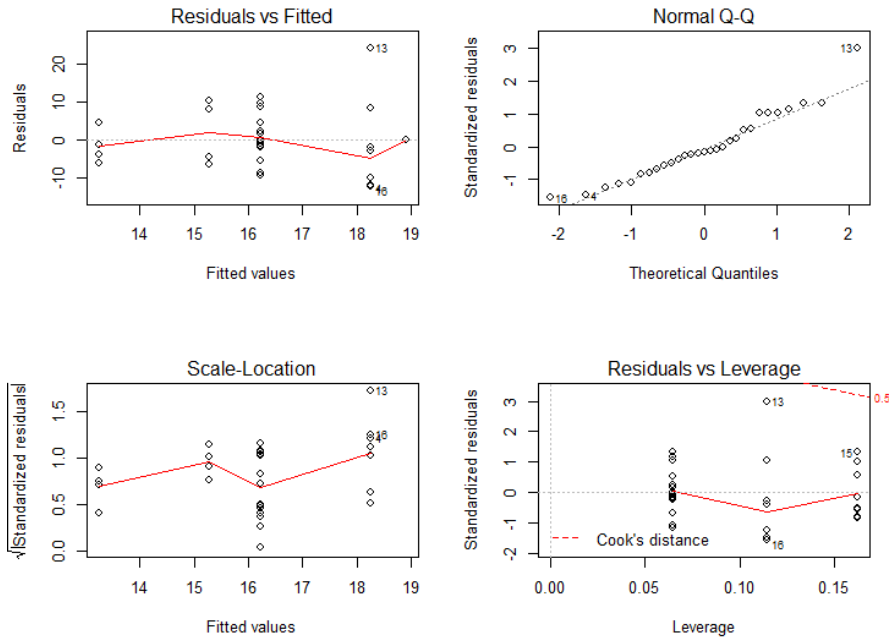


Figure 7.40: Modulus

anova_pc_modulus

• *Posterior Longitudinal*

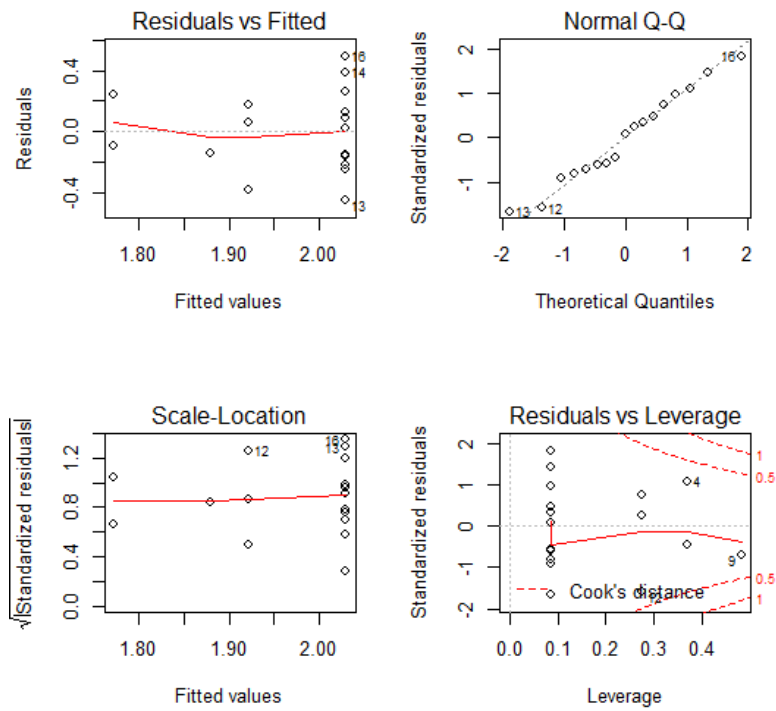


Figure 7.41: Thickness

anova_pl_thick

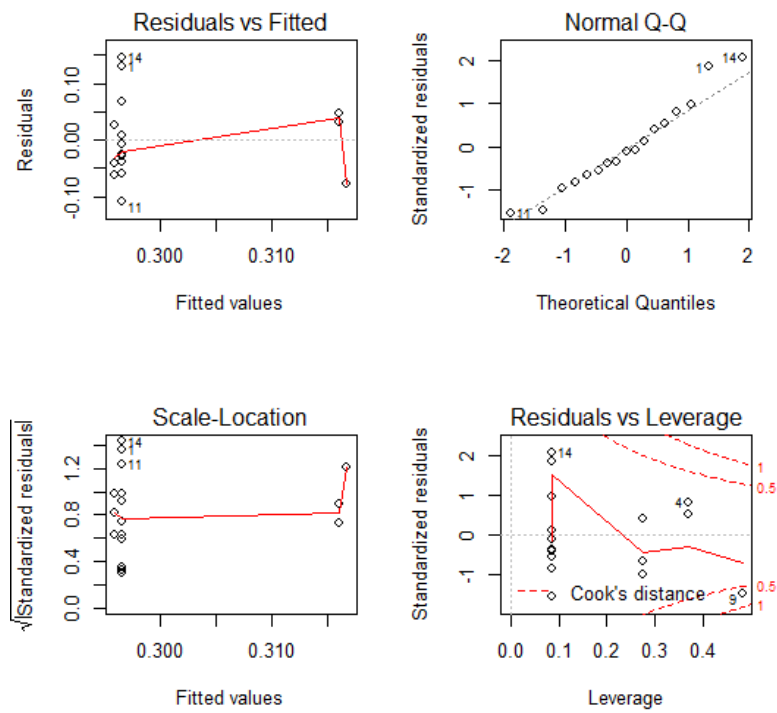


Figure 7.42: Strain at break

anova_pl_strain

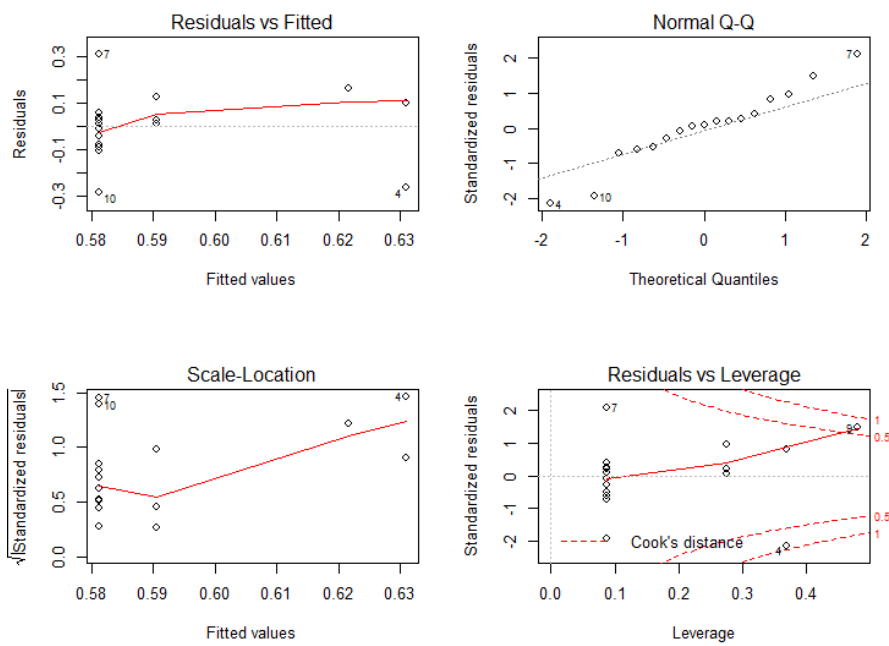


Figure 7.43: Peak stress

anova_pl_peak

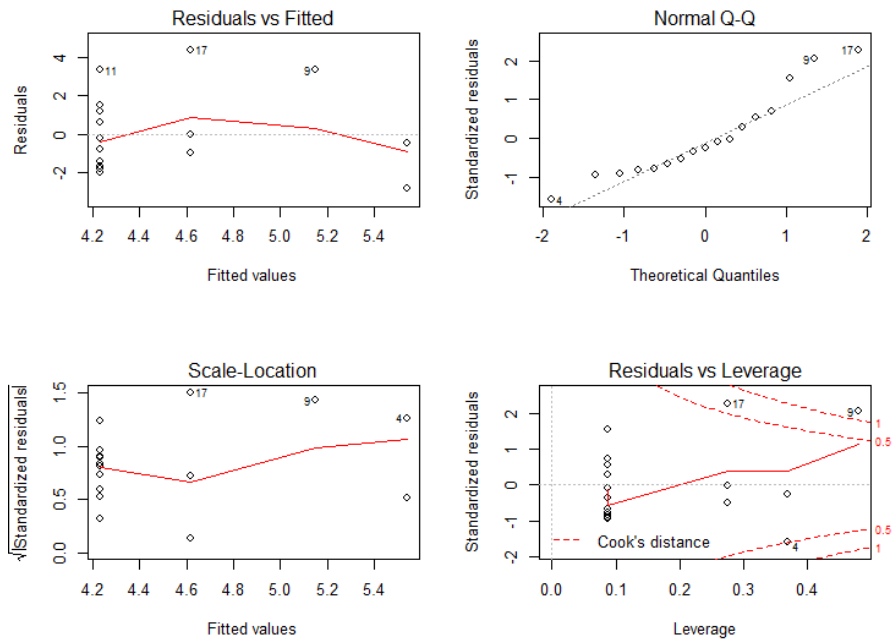


Figure 7.44: Modulus

7.3 ANOVA tables

Anova tables in output from R.

7.3.1 Two-way ANOVA tables without interaction on mean data

7.3.1

- *Anterior Circumferential*

```
> summary(aov.thicknex_ac_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category      2  0.002  0.00113   0.006  0.994
sex            1  0.174  0.17406   0.930  0.342
Residuals     31  5.803  0.18719
> summary(aov.strain.break_ac_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category      2  0.6636  0.3318   36.73 6.64e-09 ***
sex            1  0.0053  0.0053    0.59  0.448
Residuals     31  0.2800  0.0090
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(aov.peakstrex_ac_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category      2  1.149  0.5745   3.606 0.0391 *
sex            1  0.276  0.2764   1.735 0.1974
Residuals     31  4.939  0.1593
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(aov.modulus_ac_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category      2  64.5   32.26   1.781  0.185
sex            1  39.5   39.51   2.181  0.150
Residuals     31 561.6   18.11
```

AC_anova

Figure 7.45: ANOVA's results on quantities from Anterior Circumferential zone

- *Anterior Longitudinal*


```

> summary(aov.thicknex_al_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category     2  0.281  0.14056   1.285  0.292
sex          1  0.010  0.01028   0.094  0.761
Residuals   29  3.173  0.10942
> summary(aov.strain.break_al_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category     2  0.4934  0.24672  12.254 0.000139 ***
sex          1  0.0368  0.03682   1.829 0.186725
Residuals   29  0.5839  0.02013
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(aov.peakstrex_al_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category     2  0.6956  0.3478   3.228 0.0542 .
sex          1  0.0454  0.0454   0.421 0.5215
Residuals   29  3.1240  0.1077
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(aov.modulus_al_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category     2  21.4   10.708   0.837 0.443
sex          1   2.3    2.335   0.183 0.672
Residuals   29 370.9   12.791

```

AL_anova

Figure 7.46: ANOVA's results on quantities from Anterior Longitudinal zone

- *Posterior Circumferential*

```

> summary(aov.thicknex_pc_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category     2  0.1377  0.06887   0.797 0.461
sex          1  0.0465  0.04648   0.538 0.470
Residuals   26  2.2467  0.08641
> summary(aov.strain.break_pc_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category     2  0.1131  0.05654   3.053 0.0644 .
sex          1  0.0326  0.03256   1.758 0.1964
Residuals   26  0.4815  0.01852
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> summary(aov.peakstrex_pc_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category     2  0.616   0.3081   1.308 0.288
sex          1  0.498   0.4982   2.115 0.158
Residuals   26  6.124   0.2355
> summary(aov.modulus_pc_agesex)
              Df Sum Sq Mean Sq F value Pr(>F)
category     2  47.7    23.84   0.326 0.724
sex          1  27.3    27.35   0.374 0.546
Residuals   26 1899.1   73.04

```

PC_anova

Figure 7.47: ANOVA's results on quantities from Posterior Circumferential zone

- *Posterior Longitudinal*

```

> summary(aov.thicknex_pl_agesex)
      Df Sum Sq Mean Sq F value Pr(>F)
category  1  0.0959  0.09594   1.195  0.293
sex       1  0.0347  0.03471   0.432  0.521
Residuals 14  1.1238  0.08027

> summary(aov.strain.break_pl_agesex)
      Df Sum Sq Mean Sq F value Pr(>F)
category  1  0.00097  0.000970   0.176  0.681
sex       1  0.00000  0.000001   0.000  0.988
Residuals 14  0.07694  0.005496

> summary(aov.peakstrex_pl_agesex)
      Df Sum Sq Mean Sq F value Pr(>F)
category  1  0.0049  0.004919   0.208  0.656
sex       1  0.0003  0.000259   0.011  0.918
Residuals 14  0.3316  0.023685

> summary(aov.modulus_pl_agesex)
      Df Sum Sq Mean Sq F value Pr(>F)
category  1  2.97  2.973   0.577  0.460
sex       1  0.45  0.452   0.088  0.771
Residuals 14  72.12  5.151

```

PL_anova

Figure 7.48: ANOVA's results on quantities from Posterior Longitudinal zone

7.4 Two-way factorial ANOVA on unbalanced data

Here are present boxplots of all the measured data (no means) and results from R procedure for a two-way ANOVA on unbalanced data.

7.4.1 Boxplots on all data (no mean data)

7.4.1

This boxplots are plotted from the entire set of data, they should not be taken into account as a primal source of information about trends because they are not including the correlations between data that exists for measures of the same patient; however they have been plotted during computations for completeness.

- *Anterior Circumferential*

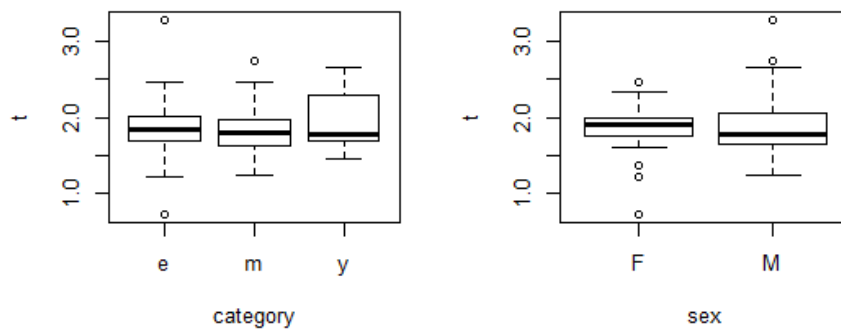


Figure 7.49: Thickness

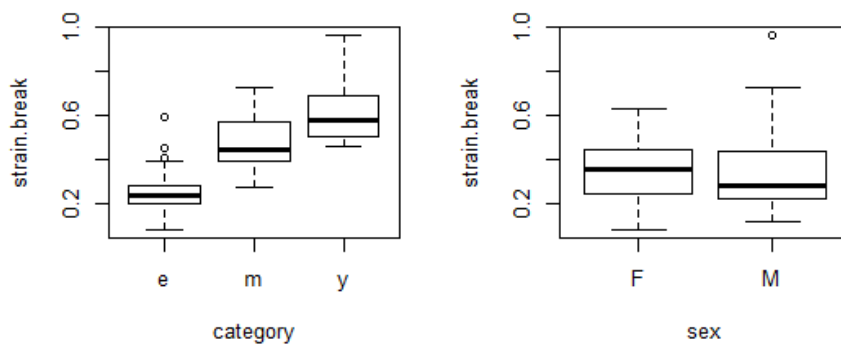


Figure 7.50: Strain at break

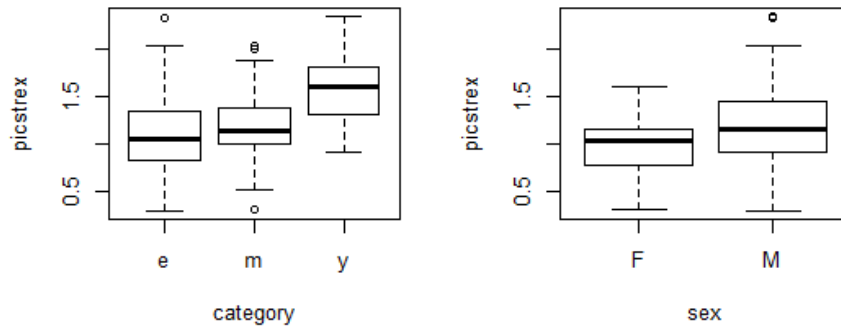


Figure 7.51: Peak stress

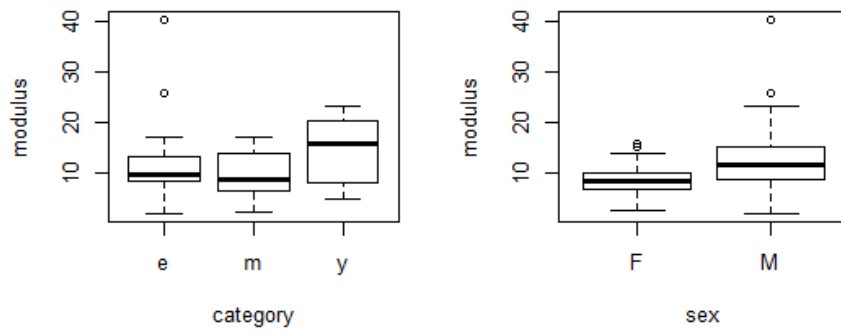


Figure 7.52: Modulus

- *Anterior Longitudinal*

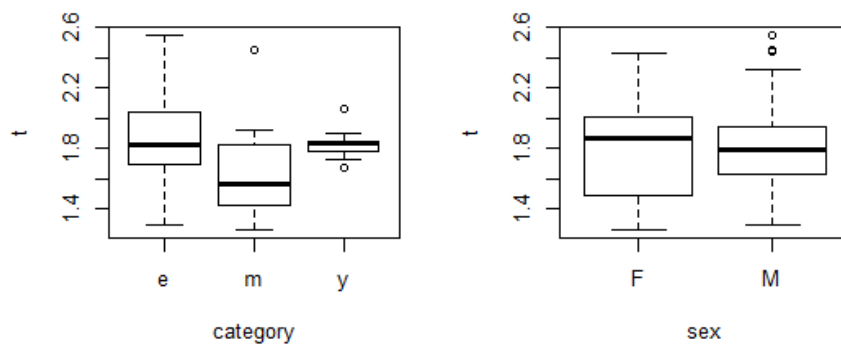


Figure 7.53: Thickness

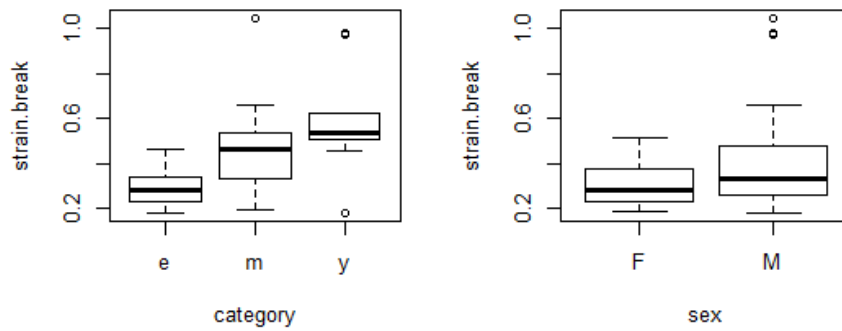


Figure 7.54: Strain at break

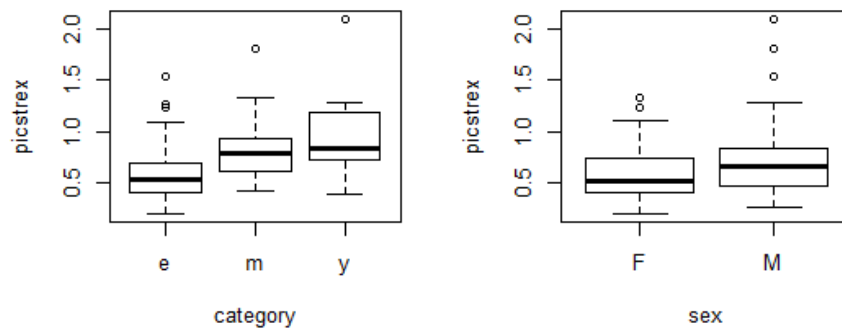


Figure 7.55: Peak stress

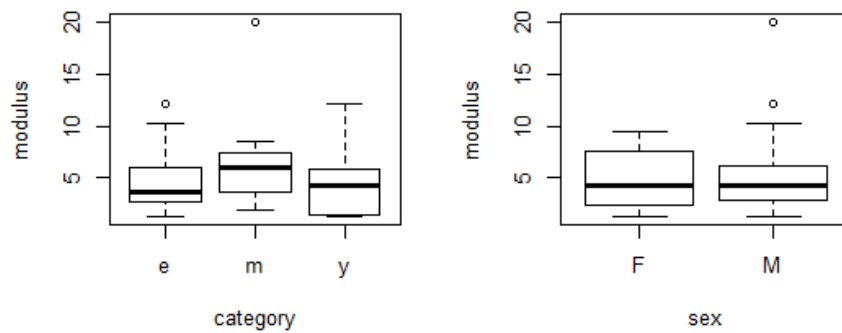


Figure 7.56: Modulus

- *Posterior Circumferential*

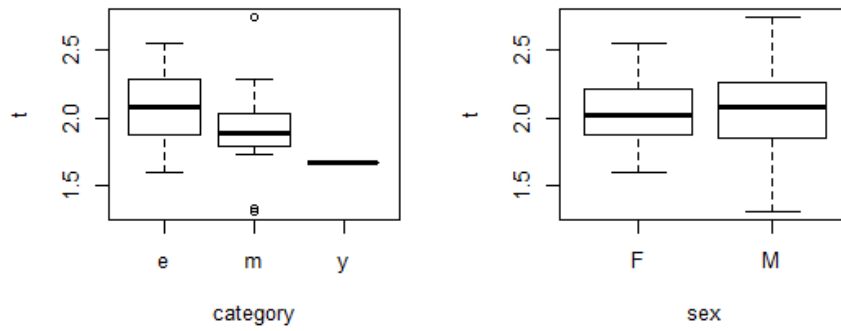


Figure 7.57: Thickness

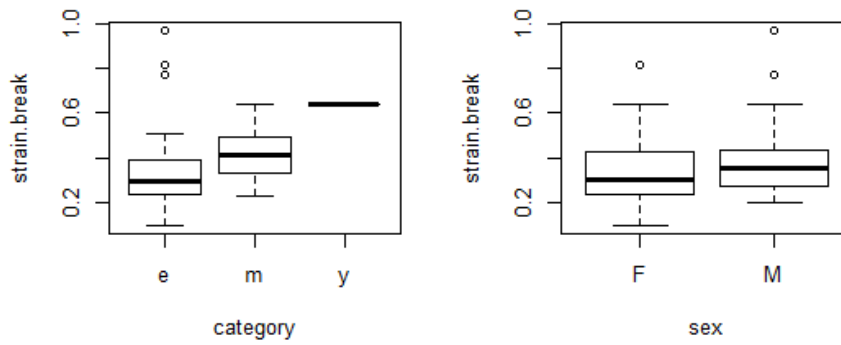


Figure 7.58: Strain at break

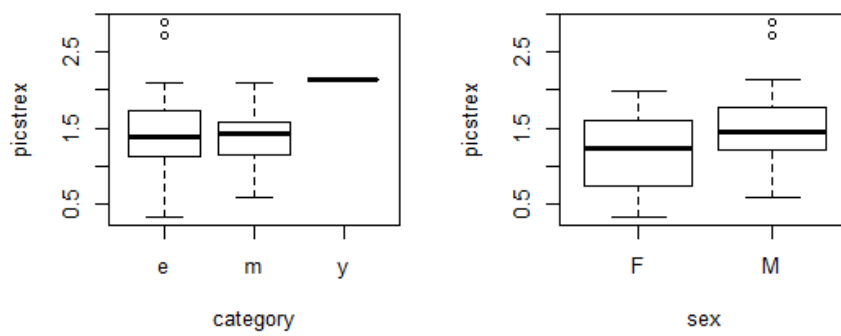


Figure 7.59: Peak stress

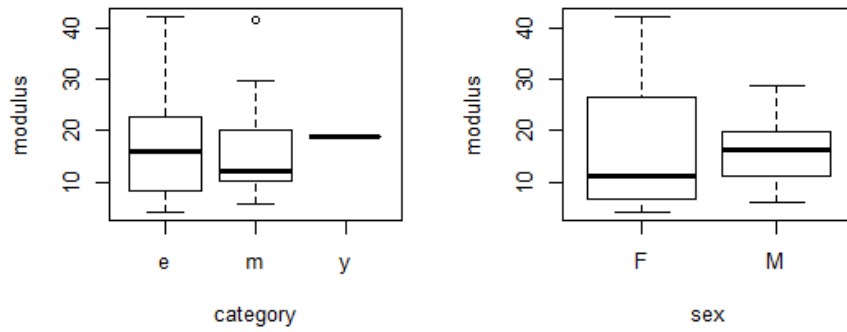


Figure 7.60: Modulus

_pc_modulus_ripit

- *Posterior Longitudinal*

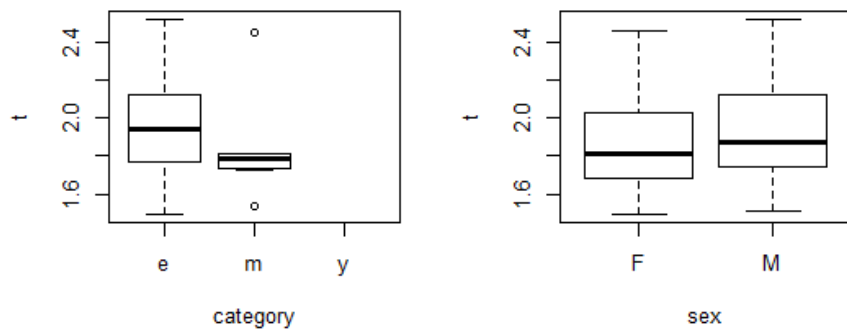


Figure 7.61: Thickness

ox_pl_thick_ripit

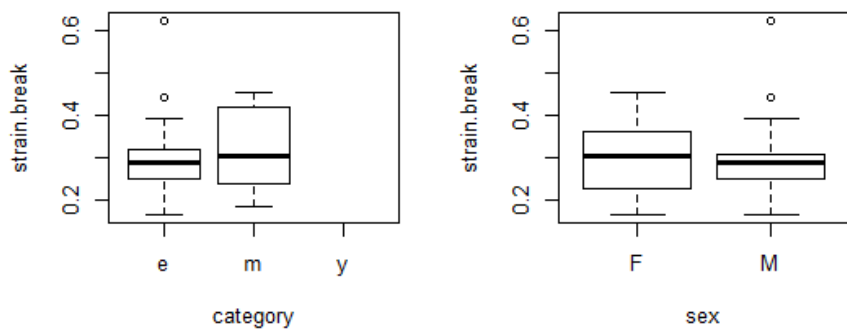


Figure 7.62: Strain at break

x_pl_strain_ripit

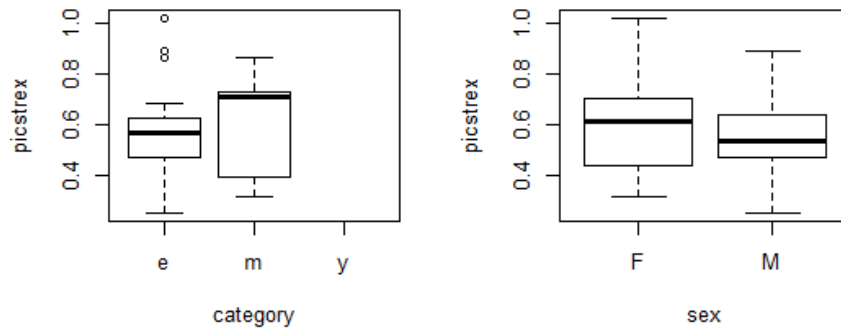


Figure 7.63: Peak stress

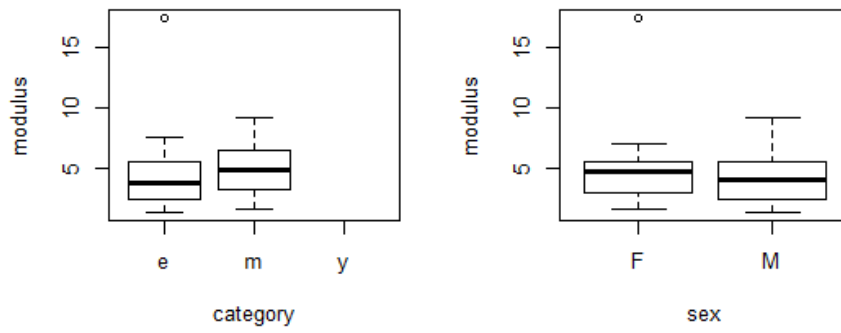
`box_pl_peak_ripit`

Figure 7.64: Modulus

`_pl_modulus_ripit`

7.4.2 Results of a two-way ANOVA-like analysis with interaction

7.4.2

Here are not present proper ANOVA tables because of the different procedure in R [27] followed. However, being an equivalent method, p-values of F-tests are present.

- *Anterior Circumferential*

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
t ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    10.856 -167.99
category:sex 1  0.59665 11.453 -165.39  4.4517 0.03795 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

> mod1 <- aov(t~category+sex, data=AC_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
t ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    11.453 -165.39
category 2  0.056318 11.509 -168.96  0.2016 0.8178
sex      1  0.030696 11.483 -167.16  0.2198 0.6405
```

(a) Thickness

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
modulus ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    2322.8 293.47
category:sex 1  20.644 2343.4 292.23  0.7199 0.3987
> mod1 <- aov(modulus~category+sex, data=AC_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
modulus ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    2343.4 292.23
category 2  55.181 2398.6 290.23  0.9654 0.38510
sex      1 156.660 2500.1 295.80  5.4818 0.02164 *
```

(b) Modulus

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
picstrex ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    13.649 -148.30
category:sex 1  0.039554 13.689 -150.05  0.2347 0.6293
> mod1 <- aov(picstrex~category+sex, data=AC_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
picstrex ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    13.689 -150.05
category 2  1.51393 15.203 -145.03  4.5344 0.01356 *
sex      1  0.95302 14.642 -146.26  5.7089 0.01917 *
```

(c) Peak stress

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
strain.break ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    0.91019 -381.17
category:sex 1  0.00072646 0.91092 -383.10  0.0646 0.7999
> mod1 <- aov(strain.break~category+sex, data=AC_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
strain.break ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    0.91092 -383.10
category 2  1.41398 2.32490 -306.52 63.6428 <2e-16 ***
sex      1  0.00594 0.91685 -384.54  0.5343 0.4669
```

(d) Strain at break

anova_ripit_thick

anova_ripit_peak

AC_anova_ripit

Figure 7.65: Results on quantities from Anterior Circumferential zone

- *Anterior Longitudinal*

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
t ~ category * sex
      Df Sum of Sq  RSS   AIC F value Pr(>F)
<none>                    5.3335 -159.56
category:sex 1  0.028608 5.3621 -161.20  0.3326 0.5662
> mod1 <- aov(t~category+sex, data=AL_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
t ~ category + sex
      Df Sum of Sq  RSS   AIC F value Pr(>F)
<none>                    5.3621 -161.20
category 2  0.62309 5.9852 -157.83  3.6604 0.03134 *
sex      1  0.00813 5.3702 -163.10  0.0956 0.75825
```

(a) Thickness

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
modulus ~ category * sex
      Df Sum of Sq  RSS   AIC F value Pr(>F)
<none>                    680.50 165.31
category:sex 1  1.8594 682.36 163.50  0.1694 0.6821
> mod1 <- aov(modulus~category+sex, data=AL_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
modulus ~ category + sex
      Df Sum of Sq  RSS   AIC F value Pr(>F)
<none>                    682.36 163.50
category 2 25.7601 708.12 161.98  1.1892 0.3112
sex      1  1.3608 683.72 161.63  0.1256 0.7242
```

(b) Modulus

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
picstrex ~ category * sex
      Df Sum of Sq  RSS   AIC F value Pr(>F)
<none>                    7.0418 -140.94
category:sex 1 0.0053025 7.0471 -142.89  0.0467 0.8296
> mod1 <- aov(picstrex~category+sex, data=AL_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
picstrex ~ category + sex
      Df Sum of Sq  RSS   AIC F value Pr(>F)
<none>                    7.0471 -142.89
category 2  1.4395 8.4866 -134.44  6.4343 0.002866 **
sex      1  0.0647 7.1118 -144.28  0.5784 0.449790
```

(c) Peak stress

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
strain.break ~ category * sex
      Df Sum of Sq  RSS   AIC F value Pr(>F)
<none>                    1.2194 -258.42
category:sex 1 0.063719 1.2831 -257.01  3.2397 0.07674 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> mod1 <- aov(strain.break~category+sex, data=AL_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
strain.break ~ category + sex
      Df Sum of Sq  RSS   AIC F value Pr(>F)
<none>                    1.2831 -257.01
category 2  0.81655 2.0997 -228.01 20.0457 1.831e-07 ***
sex      1  0.03997 1.3231 -256.95  1.9627  0.1661
```

(d) Strain at break

Figure 7.66: Results on quantities from Anterior Longitudinal zone

• *Posterior Circumferential*

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
t ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    3.6662 -120.64
category:sex 1  0.020818 3.6870 -122.36  0.2555 0.6157
> mod1 <- aov(t~category+sex, data=PC_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
t ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    3.6870 -122.36
category 2  0.47425 4.1612 -120.31  2.9584 0.06185
sex      1  0.01693 3.7039 -124.13  0.2112 0.64797
```

(a) Thickness

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
modulus ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    3744.3 225.80
category:sex 1  151.78 3896.1 225.78  1.8241 0.1836
> mod1 <- aov(modulus~category+sex, data=PC_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
modulus ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    3896.1 225.78
category 2  10.8437 3906.9 221.92  0.0640 0.9381
sex      1  3.9246 3900.0 223.84  0.0463 0.8305
```

(b) Modulus

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
picstrex ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    11.172 -64.932
category:sex 1  1.087 12.259 -62.289  4.3786 0.04206 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> mod1 <- aov(picstrex~category+sex, data=PC_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
picstrex ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    12.259 -62.289
category 2  0.41012 12.669 -64.644  0.7695 0.46913
sex      1  1.05736 13.316 -60.152  3.9676 0.05234 .
```

(c) Peak stress

```
> drop1(mod.sat, test="F")
Single term deletions

Model:
strain.break ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    1.2402 -174.84
category:sex 1  0.040021 1.2802 -175.25  1.4521 0.2345
>
> mod1 <- aov(strain.break~category+sex, data=PC_ripit)
> drop1(mod1, test="F")
Single term deletions

Model:
strain.break ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                    1.2802 -175.25
category 2  0.122078 1.4023 -174.69  2.1932 0.1231
sex      1  0.017517 1.2977 -176.57  0.6294 0.4316
```

(d) Strain at break

Figure 7.67: Results on quantities from Posterior Circumferential zone

PC_anova_ripit_thick

PC_anova_ripit_mod

PC_anova_ripit_peak

PC_anova_ripit_strain

PC_anova_ripit

• *Posterior Longitudinal*

```
> dropl(mod.sat, test="F")
Single term deletions

Model:
t ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                2.2600 -66.007
category:sex 1  0.051108 2.3111 -67.358  0.5654 0.4591
> mod1 <- aov(t~category+sex, data=PL_ripit)
> dropl(mod1, test="F")
Single term deletions

Model:
t ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                2.3111 -67.358
category 1  0.044446 2.3555 -68.806  0.5000 0.4858
sex      1  0.002129 2.3132 -69.331  0.0239 0.8782
```

(a) Thickness

```
> dropl(mod.sat, test="F")
Single term deletions

Model:
modulus ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                217.52 66.435
category:sex 1  64.036 281.55 71.918  7.3599 0.0119 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> mod1 <- aov(modulus~category+sex, data=PL_ripit)
> dropl(mod1, test="F")
Single term deletions

Model:
modulus ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                281.55 71.918
category 1  0.0098 281.56 69.919  0.0009 0.9763
sex      1  5.2361 286.79 70.452  0.4835 0.4930
```

(b) Modulus

```
> dropl(mod.sat, test="F")
Single term deletions

Model:
picstrex ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                0.77681 -96.976
category:sex 1  0.16873 0.94554 -93.275  5.4304 0.02816 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> mod1 <- aov(picstrex~category+sex, data=PL_ripit)
> dropl(mod1, test="F")
Single term deletions

Model:
picstrex ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                0.94554 -93.275
category 1  0.0001090 0.94565 -95.272  0.0030 0.9568
sex      1  0.0040065 0.94955 -95.153  0.1102 0.7426
```

(c) Peak stress

```
> dropl(mod.sat, test="F")
Single term deletions

Model:
strain.break ~ category * sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                0.25036 -129.81
category:sex 1  0.032764 0.28312 -128.25  3.2717 0.08253 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
> mod1 <- aov(strain.break~category+sex, data=PL_ripit)
> dropl(mod1, test="F")
Single term deletions

Model:
strain.break ~ category + sex
      Df Sum of Sq  RSS      AIC F value Pr(>F)
<none>                0.28312 -128.25
category 1  0.0048508 0.28797 -129.75  0.4455 0.5104
sex      1  0.0008973 0.28402 -130.15  0.0824 0.7763
```

(d) Strain at break

Figure 7.68: Results on quantities from Posterior Longitudinal zone

PL_anova_ripit_thick

PL_anova_ripit_peak

PL_anova_ripit

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