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Titolo del progetto di ricerca:

ANATOMICAL BIOMARKERS OF BODY COMPOSITION IN ONCOLOGICAL POPULATION: APPLICATION AND DEVELOPMENT OF ARTIFICIAL INTELLIGENCES

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Abstract

In the Era of precision medicine and big medical data sharing, it is necessary to solve the work-flow of digital radiological big data in a productive and effective way. In particular, nowadays, it is possible to extract information “hidden” in digital images, in order to create diagnostic algorithms helping clinicians to set up more personalized therapies, which are in particular targets of modern oncological medicine. Digital images generated by the patient have a “texture” structure that is not visible but encrypted; it is “hidden” because it cannot be recognized by sight alone. Thanks to artificial intelligence, pre- and post-processing software and generation of mathematical calculation algorithms, we could perform a classification based on non-visible data contained in radiological images. Being able to calculate the volume of tissue body composition could lead to creating clasterized classes of patients inserted in standard morphological reference tables, based on human anatomy distinguished by gender and age, and maybe in future also by race. Furthermore, the branch of “morpho-radiology" is a useful modality to solve problems regarding personalized therapies, which is particularly needed in the oncological field. Actually oncological therapies are no longer based on generic drugs but on target personalized therapy. The lack of gender and age therapies table could be filled thanks to morpho-radiology data analysis application.

INTRODUCTION

In the Era of Precision Medicine, it is necessary to solve the workflow of digital radiological big data in a productive and

effective way (1). In particular, nowadays it is possible to extract information “hidden” in digital images, in order to create a diagnostic algorithm helping clinicians to set up more personalized therapies, which are in particular targets of modern oncological medicine.

Faced with this problem, radiology images are an excellent tool to facilitate the goal to develop an integration between double-way interaction: patient-doctor-machines. Radiology is the first branch of medicine which is faced in daily clinical use, the application of software and post-processing data analysis. Probably it could be the first subspeciality able to fix the use of artificial intelligence (AI) in medicine.

Training a radiologist takes years of experience until he/she becomes useful to interact properly with machine algorithms. The information contained in a radiological study is enormous and the clinical question may require the use of information not visible to the observer's eye (2). The AI network allows us to train a digital instrument to recognize a specific pattern in images. This is done by preparing the images through standardization and convolution operations on which the network is trained to recognize the tissue to which a particular voxel belongs (3).

To date, not all potentially useful information can be extracted from radiological images using only the radiologist’s visual inspection. In fact, even the most expert radiologist’s sight is not able to detect all the information contained from the voxels of images acquired in a whole CT study because it is not possible for human beings (4). The image parameters depend on modality and consequently machine development is higher in Mega Bite composition (see table 1 below).

Table 1

Modality	Pixels	Bytes/ Pixel	Images/ Study	Data/Study
X-ray	2048 X 2560	2	3	31.4 MB
Computer Tomography (CT)	512 X 512	2	100	52 MB
Magnetic Resonance Imaging (MRI)	512 X 512	2	300	157 MB

Indeed, the digital images generated by the patient , in all radiological modality, have a texture structure that is not visible but encrypted; it is "hidden" because it cannot be recognized by sight alone. For example, a total of each CT The study can contain up to a range of 2000 grey scales corresponding to acquired Hounsfield Unit (HU) value, but 2000 greys are not physiologically perceptible by the human eye, which can only identify up to 16 grey tones (5). Even if the acquired CT images are modifiable by creating specific windows in the first analysis step, such as the parenchyma and bone window tool, many image data are not perceptible and therefore cannot be analysed.

Thanks to AI, pre- and post-processing software and generation of mathematical calculation algorithms, we can perform a classification based on non-visible data contained in radiological images. This can be made also retrospectively,

and this allows the creation of a trained network based on standard reference data already collected (6).

Among the reference data obtainable through the application of AI, especially thanks to post-processing of CT analysis, we could define the specific morphological composition for each human body, opening the field to "morpho-radiology" which is a subspecialty of radio informatics.
CT of all the modalities is the one that has more data to analyse since the human body is scanned with a thickness of less than a millimetres.

To date, there are no standard reference scales which quantify differences, scored by sex and age, in distribution of anatomical body composition. Moreover the Caucasian population is the most studied in the research process, around 80% of the scientific studies are based on Caucasian people but they are the lower number worldwide(7); we need to improve race differences due to contemporary race diaspora.
Thanks to AI, we could calculate the morphology of all the human organs and tissue distribution:

- Visceral adipose tissue VAT,
- Subcutaneous adipose tissue SAT,
- skeletal muscle SKM,
- BONE tissue volume including in the field of view of a CT study.

In the era of big medical data sharing, being able to calculate the volume of tissues, can lead to creating clasterized classes of patients inserted in standard morphological reference

tables, based on human anatomy distinguished by gender and age at least.

Furthermore, the branch of “morpho-radiology" is a useful modality to solve problems regarding personalized therapies, which is particularly needed in the oncological field.

Actually, oncological therapies are no longer generic drugs but based on target personalized therapy (8).

The variation in body composition above all the pathological condition of obesity is a significant risk factor of several cancers that imposes a substantial economic burden on Western healthcare that remains to be quantified. The excess costs and economic burden of obesity-related cancers in the US were studied by Young and all. (9) resulting that the mean incremental expenditures of treating obesity related cancer were times higher than those of other cancers and more considerable among the non-elderly cancer population (under< 65yrs both sex).

The limit of use of body mass index (BMI) and the more effective BSA (body surface area) for drug administration is well known. Furthermore, we need to be able to distinguish between male and female body composition. The creation of biometrical CT curves would in fact allow to this scope, thanks to differentiate the real anatomical compartments, differentiating them automatically or semi-automatically using dedicated post processing software. After all it could be possible to stratify obtained body composition values by gender and age. A real body composition analysis could be helpful to adjust the drugs administration; usually fat tissue is less vascularized and lower involved in drug distribution. The distribution of total fat adipose tissue (TAAT) distinguishing in visceral adipose tissue (VAT), subcutaneous (SAT) and intra

muscular anatomical compartments, is a key index of gender and age variation. To date standardized reference scales don't exist yet, while the application of this radiology imaging tool is possible thanks to AI, in fact it still in use a generic classify modality such as BMI. BMI is a very useful tool for evaluation of disorders such as overweight and obesity. However, it has the major limitation of not being able to evaluate the real body composition (i.e. VAT\SAT ratio), and it does not allow to know the distribution of body fat in each individual.

BMI is calculated as weight in kilograms divided by the height in metres squared. In adults, overweight, or pre-obesity, is defined as a BMI of 25-29.9 kg/m², while a BMI ≥ 30 kg/m² defines obesity. These BMI thresholds were proposed by a World Health Organization (WHO) expert report and reflect the increasing risk of excess weight as BMI increases above an optimal range of 21-23 kg/m², the recommended median goal for adult Caucasian population (WHO/NUT/NCD, 2000 https://www.worldobesity.org/about/about-obesity/obesity-classification).

Classification	BMI Cut-Off Points (kg/m²)
Healthy Weight	18.5-24.99
Overweight (including obesity)	≥25.00
Obesity	≥30.00
Severe Obesity	≥40.00

For adults, WHO defines overweight and obesity as follows: • overweight is a BMI greater than or equal

to 25 kg/m^2; and • obesity is a BMI greater than or equal to 30 kg/m^2

Actually, there doesn't exist a different analysis for gender or age, consequently it is mandatory to create a reference score system. BMI provides the most useful population-level measure of overweight and obesity as it is the same for both sexes and for all ages of adults (10). However, it should be considered a rough guide because it may not correspond to the same degree of fatness in different individuals. The measurement of the volume of fat or other body components is intrinsically related to the pharmacokinetics of endogenous products and drugs. AI and the development of post-processing software is therefore a valid tool to this scope. The anatomy of all human beings are constituted by the same tissue but with a different percentage volume. The application of morphology-radiology could distinguish easily the volume, with a minimum human waste time, and the body component. Fat is a fundamental anatomical biomarker, it changes with sex and age. The female human body is mainly composed of subcutaneous adipose tissue and scarce visceral adipose tissue, while the male human body has the opposite body composition; moreover aging the fat body distribution tends to change, particularly with menopause women increase VAT mean and both sexes generally increase the share of total adipose tissue in Western countries. In Western world (11), the state of health is strongly correlated to the amount of body fat accumulated and body composition, to the point that we have specific and related pathologies for obesity. In fact, cardiovascular diseases and some types of cancers are associated with the increase in body fat volume, in particular with the increase in visceral fat amount. During

the COVID-19 pandemic emergency we realized that among the very first biological risk indicators of poor prognosis there was the increase in body fat (12). In particular in the variation of distribution of adipose tissue, an increase in visceral fat compartment and VAT\SAT ratio were associated with the development of the severe form of the disease and the increased risk of hospitalization and mortality. In the past, fat adipose tissue was considered as inert tissue when in reality it would appear to be involved in the development and production of many cytokines and hormones. On pharmacokinetic point of view, on the other hand, it is an inert tissue that only limit the micro-diffusion of drugs.

To date, there are no specific gender and age scores for drug administration, but estimates based on weight and height such as BMI or more specific such as BSA, widely applied in the oncology field for administration of chemo-immuno-therapeutic drugs (13). Skeletal muscle mass (i.e., skeletal muscle index, SMI), adipose tissue, and skeletal muscle density (SMD) (i.e., a measure for skeletal muscle quality and intramuscular fat infiltration) could potentially serve as predictive covariates, as they are associated with altered volumes of distribution, metabolism, and clearance of cytotoxic drugs. Previous studies (14) demonstrated a wide variation in muscle mass and visceral adipose tissue (VAT) in patients with identical BSA and/or body mass index (BMI), producing a heterogeneity in chemotherapy tolerance and treatment-related toxicity such as neutropenia.

The microvascular network formed by the capillaries supplies the tissues and permits their function. It provides a considerable surface area for exchanges between blood and tissues. All pathological conditions cause a variation in the

microcirculation in particular the increase of total body fat. These changes can be used as imaging biomarkers for the diagnosis of lesions and optimization of treatment. Among the many imaging techniques developed to study the microcirculation, the analysis of the tissue kinetics of intravenously injected contrast agents is the most widely used, either as positive enhancement for CT, T1-weighted MRI and ultrasound – dynamic contrast-enhanced-imaging (DCE imaging) – or negative enhancement in T2*-weighted brain MRI – dynamic susceptibility contrast-MRI (DSC-MRI). The kinetics may be analysed visually, to define qualitative criteria, or with software using mathematical model to extract quantitative physiological parameters. The results depend on the acquisition conditions (type of imaging device, imaging mode, frequency and total duration of acquisition), the type of contrast agent, the data pre-processing (motion correction, conversion of the signal into concentration) and the data analysis method. Because of these multiple choices it is necessary to understand the physiological processes involved and understand the advantages and limits of each strategy. Identification of the optimal dose of chemotherapy is a science, which has been studied and validated over many years through a combination of early phase trials, randomized clinical trials, and observational studies. Drug dosing methods vary across the age spectrum, including not only traditional body surface area (BSA) formulae, but also weight
based dosing (e.g., mg/kg), flat doses, and capped doses (15). More recently, a smoothed approach to dosing in infants has been proposed that provides a dose between mg/kg and per square meter of body surface area (mg/m2).In addition to the

way in which the dose is calculated, a variety of studies have explored dose optimization (i.e., dose density, interval compression, and dose capping) to improve survival or mitigate toxicities.

MATERIALS AND METHODS

Aim study: creation of BMI-CT mathematical model correct by age and gender.

Study design: mixed (retrospective and prospective). No modification of clinical routine; no additional devices used.

Materials: Use the AI pre-trained machine learned software DAFS v3.0.2 (VORONOI Inc) on CT images study. Grouped different body shapes due to body fat distribution VAT, SAT, SKM, IMAT and BONE volume, in male and female during age. **Methods:** 500 (female , male) randomly selected patients in double blind modality i.e.: human-machine blinded.
Simple size selected in adult oncological patients presented on RIS PACS with age≥ 18 ≤90yrs). Images were acquired on CT scan. Field of view able to include severe obese patients .
Retrospective modality choosing between 1.1.2020-30.6.2020 Prospective analysis of validation: analysis of 50 patients (25 male; 25 female) using a CT scan weighted correlate to weight, height and relative body mass index calculation. It will be used as a gold standard manual segmentation (software semi-automatic). Clinical data available: weight, height and BMI index calculation, gender M/F, age scoring group, correction BSA body surface area estimation which is the best oncological method in use for drug administration. Voronoi software calculation: SKM-

VAT-SAT-IMAT (IntraMuscolarAdiposeTissue) to fully 3D measurements of these tissues and their RATIO according to the formulas VAT\SAT and TAAT(SAT+VAT+IMAT)\SKM. Comparison between 3D volume vs partitions ,in particular L3 vertebral level vs L1 and thorax vs abdomen using diaphragm as anatomical references. Secondary scope: to detect pharmacokinetics distribution of contrast agents on CT. Correlate body composition with Pharmacokinetics of contrast agent administration on CT (functional-morpho-radiology application of AI). Manually using a ROI in at least basal and venous phase, to analyse different organ enhancement and to define a K-trans of each organ (kidney cortex and medulla, liver). Kidney is needed because eliminate contrast agent, liver is chosen as reference organ for contrast parenchyma diffusion due to its unique property of double vascularity; the ROI in the liver should positioned on S7 because it is the refereed segment (less fat infiltration and less motion artifact). Descending Aorta and Inferior Vena Cava are used as models for arterial\venous phase (macro-vascularity) vs fat tissue (micro vascularity). Exclusion criteria: presence of any type of implanted metal devices (the software is no able to correct the artifact generated) Result attended: VAT\SAT (it should be higher in male than female; it should increase during age and after menopause woman should present a distribution similar to men). TAAT(SAT+VAT+IMAT)\SKM should be more in females than in male and its distribution model should decrease during age because of sarcopenia.

The primary scope is define Morpho-radiology as a helpful tool to distinguish between, male female obese\healthy and

body composition and its modification during clinical routine images acquired. The secondary scope is to create in conclusion, after a standardized contrast material injection CT-protocol with the volume of contrast material adjusted for BMI-CT model define a specific pharmacological dose distribution comparing with BSA based on real body composition analysis. AI is rapidly moving from an experimental phase to an implementation phase in many medicine areas. The combination of improved availability of large datasets, increasing computing power, and advances in learning algorithms has created major performance breakthroughs in the development of AI applications. In the last 5 years, AI techniques known as deep learning have delivered rapidly improving performance in image recognition, caption generation, and speech recognition. Radiology, in particular, is a prime candidate for early adoption of these techniques. It is anticipated that the implementation of AI in radiology over the next decade will significantly improve the quality, value, and depth of radiology's contribution to patient care and population health, and will revolutionize radiologists' workflows .

We are helping to rise and grow up a new field of radiology "Radio -Informatics" which it need a key terminology, educational needs of members research and development, partnerships, potential clinical applications, implementation, structure and governance, role of technician radiologists physics engineer the emergent needed of a specialized informatics operator. Thanks to AI the expanded repertoire of measurements from individualized tissues and organs is likely to enable the discovery of new biomarkers of aging, sarcopenia, obesity and cachexia, in particular, "subtypes" of

muscle loss and fat gain in aging and disease and contribute to greater understanding and better treatment design for the individual. Moreover we are needed to evaluate the segmentation accuracy for each tissue type

AIMS OF THE PROJECT IN RELATION TO THE REFERENCE YEAR 2021

The aim was to test the use of SOFTWARE: DAFS v3.0.2 (VORONOI Inc) supervised machine learning solution based on 3Dslicer platform trained to obtain a semantic segmentation of body composition on CT images.

METHODS USED IN THE REFERENCE YEAR 2021

VOLUME-ANALYSIS: application of 3D software to develop morpho-radiology. Subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), skeletal muscle (SKM), intramuscular (IMAT) adipose tissue and BONE volume (3D) analysis on venous scans in order to define the standard reference parameters (male vs female and scored by age).

RESULTS OBTAINED IN THE REFERENCE YEAR 2021

The software used for the aim of the study was validated, we tested the free trial version with good reproducibility measures, the entire segmentation can be reproduced in its entirety. Unfortunately, it is machine time consuming, 40 minutes for each patient. “Human time consuming” is less compared to manual segmentation which takes 20 minutes for each slice and 1h and 30minutes for obtaining the volume dataset for all the body composition parameters.

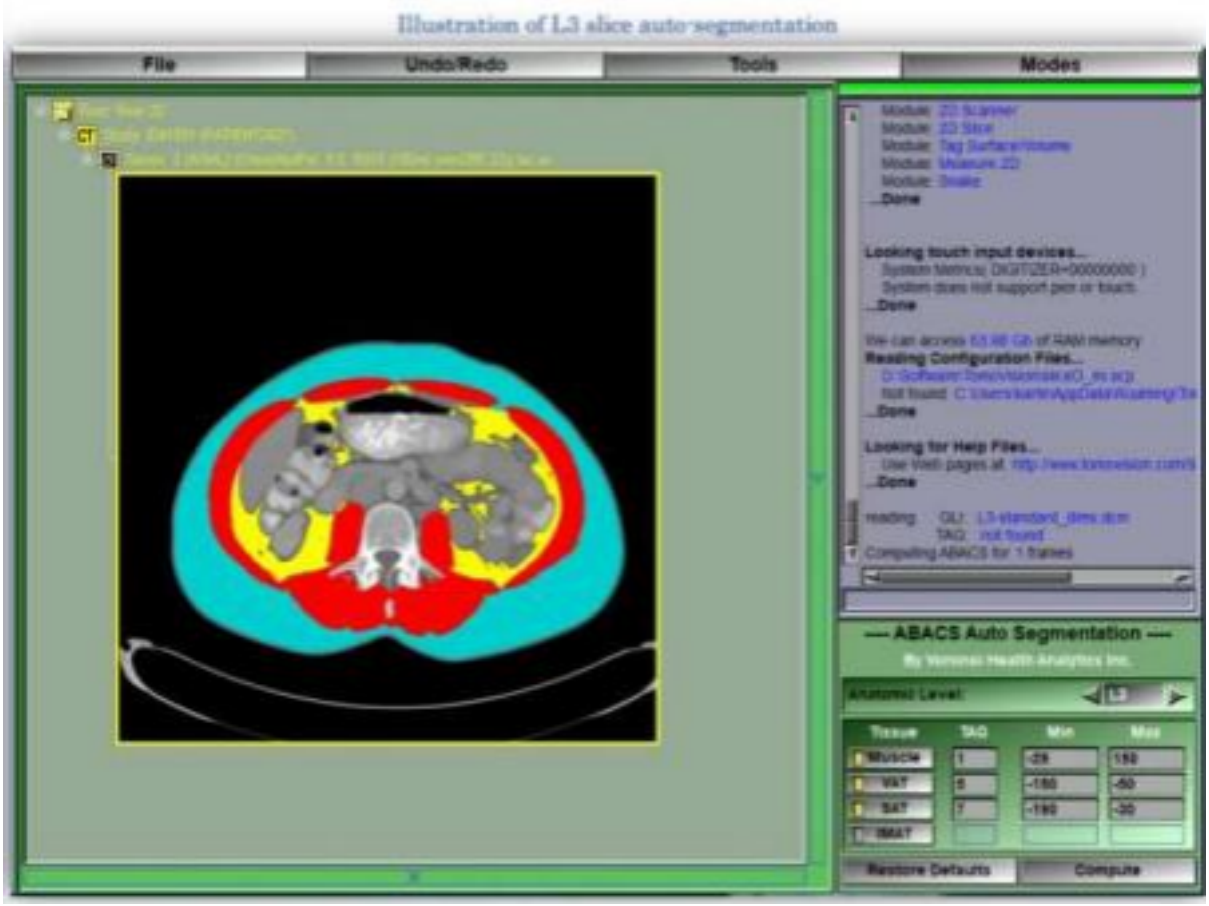
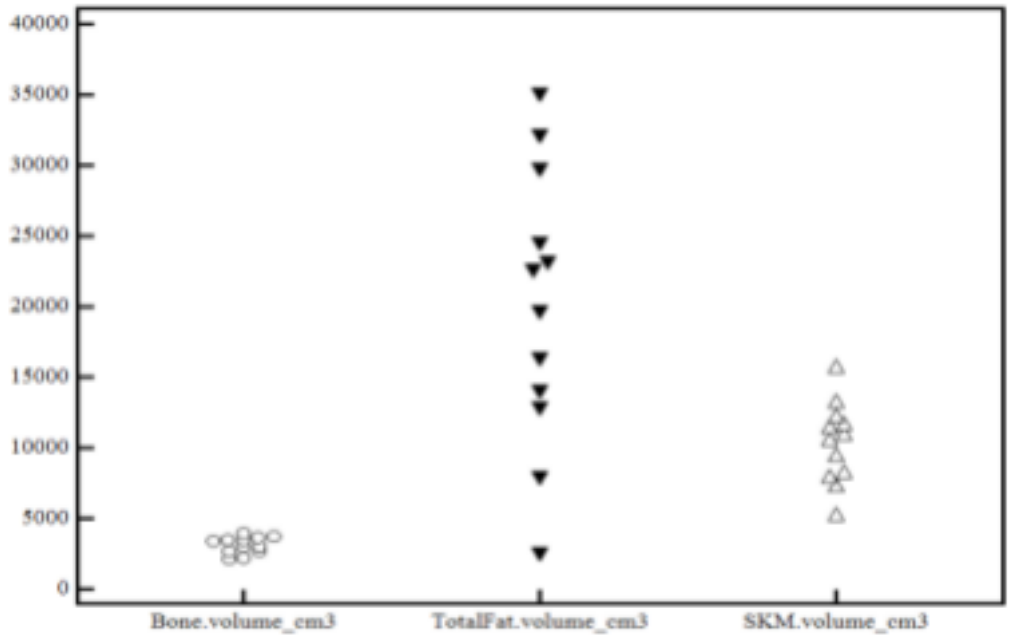


Figure 1. : Software DAFS v3.0.2 (VORONOI Inc) on CT images study (green: SAT; red: muscle SKM; yellow: VAT; blue: bone tissue)



Graph 1: preliminary result of body fat distribution calculated with VORONOI software in 12 individuals. On the axis there are the number of pixel calculated automatically

Anatomy of the fat and its compartments

In our body there is a part of primary fat, called "essential fat", which represents the amount of fat contained in central nervous system, bone marrow, mammary glands, kidneys, spleen and other tissues (16). Considering the particular anatomical localization, essential fat has a physiological role of primary importance; it is considered the minimum percentage of fat mass compatible with a state of good health. When the percentage of body fat decreases to only the amount of essential fat or a little more, the organism suffers; for example, there is a greater susceptibility to infections in men and amenorrhea woman, often accompanied by alterations in bone metabolism and osteopenia, which is typical condition in women suffering of anorexia(17). The storage fat added to the primary fat gives us the amount of total body fat and is concentrated in the subcutaneous, thoracic, abdominal (where it is called

"visceral"), intra and intermuscular sites. Visceral fat, also known as abdominal fat because it is localized under the diaphragm, is the part of adipose tissue concentrated within the abdominal cavity and distributed as support between the internal visceral organs. Visceral fat differs from subcutaneous fat which is concentrated in the hypodermis (the deepest layer of the skin) and from intramuscular fat, which is instead distributed between muscle fibres. As for its role, in previous time adipose tissue was considered an inert organ, with a function only in the global energy homeostasis of the organism (18). Moreover, it was believed that its function, in addition to providing thermal and mechanical insulation, was only to store excess energy in the form of triglycerides with high calories, and then to give it back, as needed, as free fatty acids in blood vessels. A revolution point of view has been going underway for just over a decade in the way of understanding the biological functions of adipose tissue. Currently it is seen as a dynamic organ, involved in a wide range of biological and glyco-metabolic processes, it has also complex interactions with the brain and peripherical organs. This different perspective was imposed by the discovery that adipose tissue is now seen as an endocrine organ (19). In fact, adipocytes secrete a series of hormones and cytokines, factors and protein signals, called adipokines, which are associated with the role of the adipocyte in energy homeostasis and contribute major complications that accompany diseases such as obesity. The total number of adipokines, many documented and some putative, now exceeds a number of 50. Each category of adipokine corresponds to a specific function within the organism. In addition to intervening in the regulation of body weight, leptin regulates puberty and reproduction,

placental and fetal functions, immune response and sensitivity to muscle and hepatic insulin. In fact, in patients with lipoatrophy, (i.e., lack of adipose tissue), causes severe hypo-leptinemia, which is associated with severe insulin resistance, hepatic steatosis and dyslipidemia. On the other hand, the same hyperleptinemia, typical of most obese people, seems to have a pro-atherogenic role by contributing to insulin resistance, altering endothelial function, favouring the onset of atherosclerotic disorders and platelet aggregation and arterial thrombosis. The detection of leptin marked a major revolutionary in our understanding the endocrine role of adipose tissue. The roles of leptin in energy homeostasis, glucose and lipid metabolism, and immune and neuroendocrine function have been shown in humans congenital or acquired leptin deficiency (e.g., fasting and lipodystrophy).The term adipokine was coined to generically identify all the molecules synthesized and secreted by adipose tissue. Their mechanism of action is both paracrine and endocrine. Leptin thus regulates multiple bodily functions, its expression and leptin secretion increases in obesity, probably due to increased visceral fat. As adipocyte surface area increases in obesity, there is increased expression of leptin, interleukin 6 (IL6), IL8, monocyte chemoattractant protein 1 (MCP1), and granulocyte colony-stimulating factor. These and possibly other cytokines attract pro-inflammatory macrophages (M1 type), which release factors such as tumor necrosis factor α (TNFα) that may have local and systemic inflammatory effects (30). Adiponectin, on the other hand, has a role in the regulation of energy metabolism, favours the oxidation of triglycerides and increases the insulin sensitivity of muscles and liver; the expression and secretion of

adiponectin conversely decreases in obesity. Adiponectin is richly expressed in adipocyte but, unlike other adipokines, its plasma levels are reduced in abdominal adiposity; visceral adipose tissue (VAT) is likely to produce a factor that inhibits adiponectin synthesis, which some people identify as TNF-α. Furthermore, adiponectin is shown to have important antiatherogenic, antidiabetic and anti-inflammatory properties in humans. Subjects with elevated plasma adiponectin levels have a significantly reduced risk of major cardiovascular events, even after adjustment for low- and high-density lipoprotein-related cholesterol, body weight, diabetes, and arterial hypertension. According to some authors (20), the hypoadiponectinemia that characterizes subjects with abdominal obesity and metabolic syndrome would be the key element to justify the cardiometabolic risk of this situation. Visfatin, recently added to the list of adipocyte-derived factors, appears to be specific to abdominal fat deposits; in fact its plasma concentration correlates with the degree of abdominal obesity. Visfatin has effects similar to those of insulin; it is thought to activate the insulin receptor by binding it to a point distinct from that of the insulin. An adipokine, which plays a very crucial role, is leptin; various experiments on mice, with mutation of the gene for leptin, have shown an increase in obesity in association with an altered thermogenesis and severe insulin resistance, typical of the metabolic syndrome (21). Adipose tissue is also an important site of angiotensinogen and angiotensin II production. Higher levels of mRNA for angiotensinogen are detectable in the adipose tissue of obese subjects compared to that of normal weight subjects, and a positive correlation between plasma levels of

angiotensinogen and adiposity is detectable. It was therefore assumed that the increased synthesis of angiotensinogen and angiotensin II may contribute to hypertension to explain the frequent association with obesity. In conclusion, an essential breakthrough in understanding the role of visceral adipocytes was determined by the recognition that VAT is the pathogenetic site of many of the disorders related to obesity. In 1993, Hotamisligil et al. demonstrated an increased expression of TNF-α in the adipose tissue of the genetically obese animal (22). The idea that a factor produced by adipose tissue was involved in the genesis of insulin resistance at that time was revolutionary, actually it is a certainty. Since then, many other factors secreted by adipose tissue have been identified such as interleukins (IL-1, IL-6, IL-8, IL-10), interferon-γ, growth factors such as transforming growth factor-β and the growth factor vascular endothelial growth, chemotactic proteins (chemotactic factor for monocytes-1) and complement cascade factors. The circulating levels of these factors increase with the increase in fat mass, especially if located in the abdominal area.

Hormonal differences due to sex, android and gynoid obesity

In 1950 Jean Vague introduced the distinction between "android" obesity and "gynoid" obesity, noting that the android was associated with a greater risk of hypercholesterolemia, hyperuricemia, hypertension, and reduced carbohydrate tolerance (23). In addition, moreover , from a quantitative point of view, obesity should also be investigated from the qualitative point of view. In physiological conditions, male and female are distinguished by a different distribution of the adipose mass, indeed the

body forms are linked to the relationship between male (androgenic) and female (oestrogen) sex hormones. This phenomenon becomes evident during post-menopausal period, in which, due to the drop in oestrogen levels, there is a redistribution of body fat composition in women characterized by an increase in VAT. Nevertheless, it is in a pathological condition that the man-woman differences on the distribution of the adipose mass become accentuated, giving rise to the two main types of obesity: android obesity and gynoid obesity. The first one is typically male, it is associated with a greater collection of fat mass in the abdominal, dorsal and cervical-nuchal region. Furthermore, there is a significant intra visceral deposition in android obesity phenotype. Regarding gynoid obesity, when it occurs in women, the distribution of fat follows the so-called "pear shape": greater in the lower part of the abdomen below the waist, in the gluteal and femoral regions. Contrary to android obesity, fat is predominantly represented in the subcutis. Android obesity is more dangerous in its association with cardiovascular complications such as strokes and heart attacks as a pivotal role in the metabolic syndrome. For example, individuals with high levels of visceral fat , central obesity or android shape, are clearly more insulin resistant and at higher risk for Type 2 diabetes mellitus (T2DM) than those with high levels of peripheral, mainly subcutaneous, fat. Body measurements can be made clinically quickly with a simple tailor meter that reveal the type of obesity. Even if anthropometric measurements are different and susceptibility by operator error, through a ratio between the circumference measured at the umbilical level (waist) and the circumference at the level of the buttock (hips), called WHR (from the

English Waist to Hip ratio) we can clinically speak of android obesity if this ratio is greater than 0.85. While it is a question of gynoid obesity if the ratio is less than 0.79. However, all men who have not a WHR below 0.95 and all women who have not a WHR below 0.80 are considered to be at high risk for developing obesity-related diseases (24). Regarding the development of pathologies associated with the two types of obesity, it can be argued that android obesity is frequently associated with T2DM, dyslipidemia, cardiovascular diseases and hyperuricemia; conditions which are grouped under the term "multi metabolic syndrome" and which represent a serious risk to the patient's health (29). Experimental investigations have shown that visceral or internal adipocytes are more sensitive to the lipolytic ("slimming") activity of some hormone the catecholamines. When adipose and non-adipose molecules (adipocytes also release hormones and substances with a pro inflammatory action) coming from the metabolism of visceral fat reach the liver, they "flood" it and alter its functioning. The modification of hepatic metabolism causes alteration of many blood values and therefore the onset of hyperinsulinism/insulin resistance (type II diabetes and cardiovascular diseases (hypertension, dyslipidemia, myocardial infarction). Research has shown that even in people of normal weight and overweight there can be significant accumulations of fat around internal organs (31). Even apparently thin individuals can therefore be exposed to an increased risk for all the pathologies traditionally associated with android obesity (25). However, it is noteworthy that recent imaging studies, including the Framingham Heart Study, have highlighted not only the importance of visceral adipose tissue, but also other ectopic fat

depots such as liver or renal fat (33) .The BMI is a widely used parameter to obtain a general assessment of one's body weight that reveals whether an individual is classified in an area of weight which can be: normal - underweight - overweight - medium obesity degree - high-grade obesity. The index is obtained through the mathematical formula that divides the weight in kg of the subject with the square of the height expressed in meters. Statistical studies have shown that the BMI value to be associated with lower morbidity and mortality for all diseases for which being overweight is a risk factor (cardiovascular disease, diabetes, hypertension, osteoarthritis, and some neoplasms) is between 20 and 25. On the other hand, the optimal BMI value for an individual between 19 and 35 years old is 22 (range of value 19-25 kg/m^2); while for an individual over the age of 35 it is very variable.

Population-based actuarial studies place the upper limit of normal BMI in adults at 25 kg/m2, define obesity as a BMI > 30 kg/m2, and designate a BMI between these values to be "overweight." The degree of obesity can be further subcategorized into class 1 (BMI of 30 to <35), class 2 (BMI of 35 to <40), and class 3 (BMI of >40) (26).

It is mandatory to consider for each single patient anamnesis, age, and gender, known dietary and the presence of other physical conditions which could modify his performance clinical-nutritional status. Previous studies of SM Chan, have tried to correlate idiopathic diseases such as Crohn's disease or ulcerative colitis to the presence of a high BMI, as it is thought that obesity could play an important role in the development of a pro-inflammatory state which in turn could be involved in the aetiology of inflammatory bowel disease (34). However, recent studies, BMI and the Risk for Crohn's Disease

and Ulcerative Colitis (both diseases known as inflammatory bowel disease IBD) and the high prevalence of overweight and obesity in adults with Crohn's disease. Associations of disease and lifestyle factor study did not support the correlation between obesity and Crohn's disease – ulcerative colitis (27). On other hand, an opposite thesis, which is being explored lately, sees fat as a protective role for diseases such as Crohn's disease. In the article "Visceral adipocytes: old actors in obesity and new protagonists in Crohn's 'disease" it is shown that visceral adipose tissue increases by 27% in patients after being treated with infliximab; indeed, this would go against the idea of adipose tissue seen as a risk factor for the development of Crohn disease (28).

Brown fat: role and development

The adipose deposits in the mammal are composed of two basic cytotypes, the white and brown adipocytes; their respective quantity depends on species, genus, age, and environmental and nutritional conditions. The mature white adipocytes, with a thin cytoplasmic layer and nucleus squeezed at the periphery by the central collection of triglycerides, were for a long time considered only deposits of high energy molecules, capable of supplying fuel to the other cells of the body during fasting. Brown adipocytes have an almost central, round nucleus and large mitochondria, with small drops of triglycerides scattered among the cytoplasm; they have a totally different role from white adipocytes, being responsible for thermogenesis. In these cells the fatty acids, instead of being circulated to supply energy to other tissues, are removed from the surface of the

lipid droplets and oxidized by the mitochondria; this mechanism allows energy to be dissipated in the form of heat instead of producing ATP. It is known that almost all. the adipose organ in adults is composed of subcutaneous and visceral deposits of white adipose tissue, while brown fat is particularly abundant in the new-born and constitutes 3-5% of the total weight. This fat is richly vascularized and innervated and is in some particular areas of the body such as the base of the neck, around the heart and kidneys, the adrenal glands and between the shoulder blades. Primary brown fat cells begin to differentiate from the 26th to the 30th week of gestation and increase in size and number from 3 to 6 weeks after birth. Its role in the new-born is of considerable importance, as the production of heat in the adult subject occurs mainly through voluntary and involuntary muscle activity (shivering or tremor), while in the new-born these mechanisms are not fully developed and are replaced by brown fat with its function of chemical thermogenesis, that is the production of heat, thus preventing the lowering of body temperature.

Therefore, taking into account the differentiation times of the primitive brown fat cells in the new-born, it can be understood how the thermic dispersion of heat represents a not negligible danger for the new-born. Similarly, even in those with damage to the central nervous system, or suffering from hypoglycaemia, they have more difficulty in maintaining body temperature. This evidently finds its rationale precisely in the alterations of the thermoregulatory centres of the central nervous system.

More discussed are the presence and role of brown adipose tissue in adult humans; it was common opinion that this,

unlike the new-born, was absent and that in the adult thermogenesis, not linked to muscle activity, was not necessary or that it was supported by other mechanisms. This belief has been disproved by random surveys, increasingly frequent with the spread of positron emission tomography with fluoride-deoxy glucose, (PET/CT) of hypercaptating areas, not attributable to pathological tissue, but due to the metabolic hyperactivity of collections of brown fat. Several institutes have come to this conclusion after having investigated the issue, such as the University of Maastricht in the Netherlands, where a research involved 24 young adult males (10 of normal weight and 14 overweight) treated with radioactive glucose and subjected to a temperature of 16 ° C. Performing PET/CT scans revealed deposits of brown adipose tissue in the neck, chest and abdomen in 23 individuals under examination; because the brown adipose tissue was activated in response to the body's exposure to cold. Furthermore, another data emerges from this research which would reveal that the activity detected by PET/CT is greater in normal weight subjects.

A second research group, at Harvard Medical School in Boston, evaluated the presence of brown adipose tissue by subjecting nearly 2,000 patients to PET/CT. About 5% showed this tissue although the researchers did not pre-chill the subjects to the investigation. Women appear to have more brown adipose tissue than men and a decrease in brown fat was observed with increasing age. In a third study, researchers from the University of Gothenburg in Sweden used PET/CT to locate more accurately brown fat deposits in a group of volunteers brought to low temperatures to increase tissue activity. PET could be a perfect useful modality for evaluation

of biomarkers of higher circumference of the neck to better differentiate type of obesity

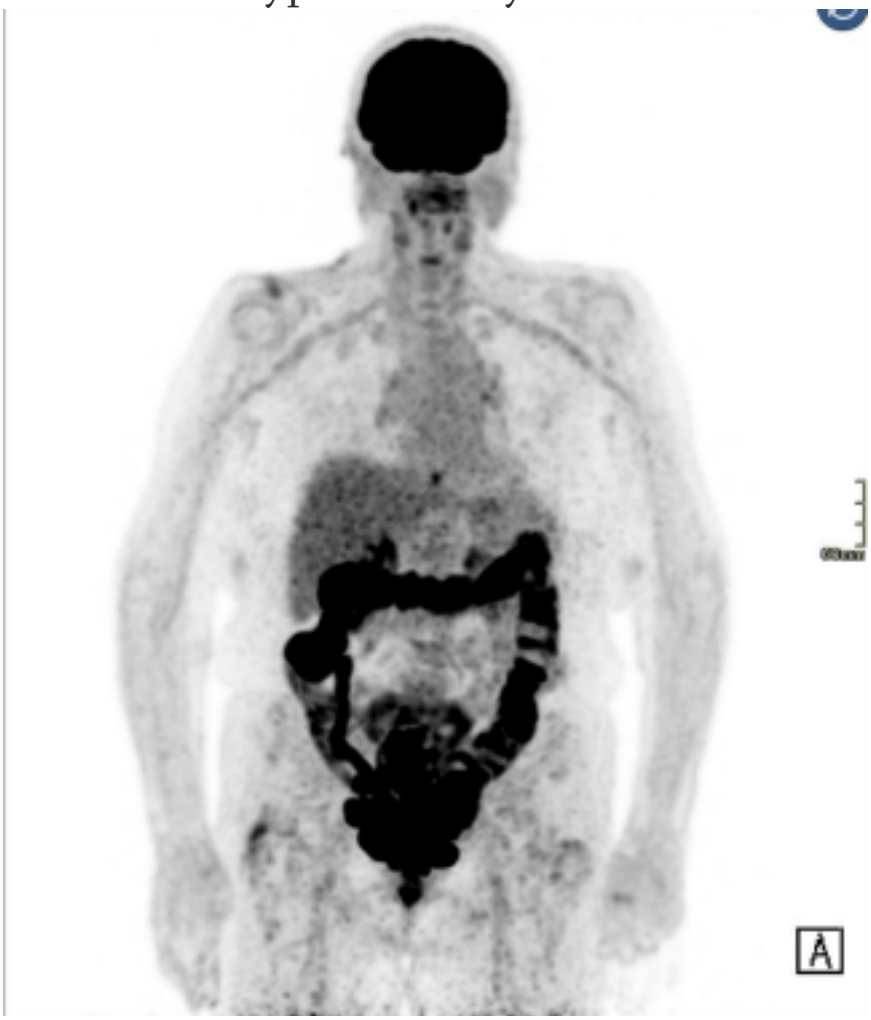


Figure 1: PET-CT is often positive in nuchal region area, consecutively it is not an indicator for brown fat. But neck circumference higher is well known to be a clinical indicator during routine practice for many diseases such as Obstructive Sleep Apnoea Syndrome OSAS.

Abdominal fat tissue

CT, especially in the abdominal region, is a very common type of investigation for a very wide range of medical conditin during clinical routine. In addition to the main purpose for which a CT scan is prescribed, which varies from case to case,

it can be used to obtain a large amount of additional information on risk factors or other diseases: the approach for which data are also processed and give this secondary information is called opportunistic screening modality. In this chapter the advantages of this approach are presented from different points of view, through some studies that highlighted the partial inadequacy of the protocols currently used in the evaluation phase of the patients' body composition. Obesity is an extremely widespread condition that can be treating by intervening on numerous risk factors, the most important of which is diet. It is estimated that 2.1 billion people worldwide are obese or overweight, with an annual worldwide healthcare cost of 2 trillion, so it is very important to fight or at least limitate this condition, in order to do it is important to estimate the degree of obesity, and scoring it by gender and better by race (35). The worldwide prevalence of obesity has doubled during the last 50 years, and according to the World Obesity Federation, one third of the people on Earth will be obese by the year 2025 (36). As seen above the most important and widespread indicator currently in use to estimate the amount of fat in a person is the body mass index (BMI). Obesity is configured, according to this scale, when the BMI exceeds 30 kg/m^2, while overweight is between 25 and 30 kg/m indipendent from gender[2]. BMI is not always an accurate index, in reality it does not take into account muscle mass, which affects weight by increasing BMI, and fat anatomical distribution, which affects the severity and quantity of adverse effects of obesity. Moreover an android/central-type fat distribution is associated with a higher risk than a gynoid-type distribution, so for the same BMI and muscle mass, an

obese man or a woman with central adiposity will have a worse risk profile than an obese gynoid woman. The need in this case is to find a more precise method than the BMI to estimate the degree of obesity.

The abdominal visceral fat obtained from a CT examination is strongly correlated both to BMI and to the waist circumference to hip ratio which is another clinical parameter quickly used for a rough estimate degree of obesity; however, there is an even greater correlation of abdominal visceral fat, compared to the aforementioned measure, with typical conditions of obesity such as hypertriglyceridemia, hyperglycemia and hypertension and metabolic syndrome. The measure of visceral abdominal adipose tissue (VAT) is usually performed on a CT images at the height of the L3 vertebra level or in the intervertebral spaces between L2-L3 (37). This abdominal area is believed to reflect, in most studies, the tissue composition of the entire body, especially at the level of skeletal muscles and adipose tissue. This is very useful because it allows us to perform a body composition estimation starting from a single slice (38). Adipose tissue has HU negative values between –190 and –30 in the regions mentioned above. An abdominal visceral fat surface greater than 100 cm^2 corresponds on average to a BMI greater than 25 kg/m^2, while obesity appears to occur more frequently for abdominal visceral fat values greater than 130 cm^2. Finally, given its close correlation with obesity indicators, large abdominal visceral fat values are associated with increased mortality.

Considering all these factors, always in the perspective of opportunistic screening, it is convenient to use the information coming from the CT to develop the best plan,

both in terms of controls and analyses in general, given the wide range of problems that obesity condition implies.

Besides bone and adipose tissue, one last tissue that is important to quantify is muscle tissue, especially for the assessment and control of "sarcopenia", a medical condition characterized by reduced muscle mass, often accompanied by reduced muscle power typically occurring in an oncological condition. The muscle mass loss process that leads to sarcopenia is unstoppable and accelerates with aging, but it can be controlled by intervening on diet and lifestyle.

Age-associated obesity and sarcopenia are closely connected and are reciprocally regulated by adipose tissue and skeletal muscle dysfunction. During ageing, adipose inflammation leads to the redistribution of fat to the intra-abdominal fat tissue and fatty infiltrations in skeletal muscles, resulting in decreased total weakness and functionality (40).

A large number of diseases are associated with sarcopenia, including depression and autoimmunity, renal, cardiovascular, hematological and neurological diseases, resulting in significant health costs and increased mortality.

DEXA (Dual Energy X-ray absorptiometry) has also been the standard for muscle mass estimation for a long time, with CT used mostly for research purposes. As for adipose tissue, the assessment of muscle mass is carried out by measuring the surface (cross sectional area, CSA) of muscle tissue at the height of the L3-L4 intervertebral space or alternatively at the level of the L3 vertebra, allowing to calculate the "skeletal muscle index", defined as the ratio of the surface area in square centimeters of muscle tissue to the patient's height in square meters. The range of the Hounsfield scale used in most studies to identify muscle tissue in the aforementioned

regions lies between –29 and 150 HU. Although there is no direct evidence that an early diagnosis of sarcopenia can lead to better patient health outcomes and lower healthcare costs, it is well understood that a change in diet and lifestyle often slows down muscle loss and an assessment of the degree of sarcopenia can help in decision-making processes, especially in cases where the patient is undergoing oncological therapies.

FIRST TEST: Body composition assessment

Methods for assessing human body composition have been developed and validated for research focused on aging and chronic diseases, such as obesity , cited Cosqueric, et al. 2006. Among the methods that are generally considered standard in the assessment of human body composition we have Dual Energy X-ray Absorptiometry (DEXA), magnetic resonance imaging (MRI) and computed tomography (CT).

In addition, to measure body composition in clinical populations there is also Bioelectrical Impedance (BIA), an inexpensive, practical, and accessible method (41).

Although BIA is safe and non invasive, it does not expose patients to radiation, but it may not have the specificity and accuracy of DEXA, MRI, and CT.

While MRI and computed tomography allow for the precise segregation of individual tissues, CT imaging, with its excellent resolution of adipose tissue and skeletal muscle, provides an ability to test the implications of these distinct attributes in cancer patients. In addition, the layer thickness that can be acquired with modern CT equipment is much thinner than MRI, even the most performing ones. Regional analysis of adipose and lean tissue is highly correlated

with the corresponding compartments of the whole body and can provide precise quantification of specific adipose tissues, skeletal muscles, and organs. CT imaging is essential both to maximize the probability of survival and to ensure the best possible quality of life in case of survival. For these reasons, CT is the mode of choice for diagnosing and following cancer patients in most current patterns of international clinical practice. Over the past decades, many measurement techniques and equations have been developed for body composition assessment; among them most accurate method fo some authors to determine body fat is bone mineral density (double X-ray absorptiometry, DXA) (43).

DEXA, on the other hand, accurately represents whole body fat mass (FM) and fat free mass (FFM), has regional analysis capability, and is widely used to assess skeletal muscle based on limb analysis (42). The latter, therefore, provides high precision, but is not readily available in cancer centers.The estimation of body composition is essential for cancer patients to define a therapeutic plan, especially to have a correct dosage, as chemotherapy has systemic effects both in the short and in the long period of time.

The definition of personalized therapeutic plans based on the body composition of each patient should pass almost exclusively through imaging, especially CT, and it is essential not only to maximize the probability of survival but also to ensure the best possible quality of life.

A study during 2020 by Franzoi et al. compared different parameters for estimating body composition, demonstrating a certain "protective" effect of obesity known as obesity paradox. Presently, several epidemiological studies demonstrate positive associations between the prevalence of

obesity, as judged by increased body mass index, cancer-related incidence, and mortality (44).

In contrast, involuntary weight loss, which involves loss of muscle and fat, often accompanies advanced disease, and is associated with poor response to treatment and reduced survival. This is because the compartments of fat and lean tissues represent the distribution sites of lipid and water-soluble drugs, respectively, and are probably decisive for the efficacy and toxicity of chemotherapy (Prado et al. 2007).

Furthermore, body weight and weight loss vary considerably in their composition. Simultaneous loss of skeletal muscle and an increase in adipose tissue can occur, culminating in the condition of sarcopenic obesity.

The latter is characterized by both a reduction in muscle size and an increase in the proportion of intermuscular and intramuscular fat (Delmonico MJ, et al. 2007); therefore, the infiltration of fat can be a further manifestation of the wasting process. Sarcopenia was related to lower PFS (progression-free survival) but also to greater sensitivity to therapy toxicity, which is why a careful assessment of muscle mass in this type of patient could make a difference when it comes to therapy to be adopted.

It is important to emphasize that the protective effect of obesity should only be understood as a greater efficacy of inhibitor therapy in overweight and obese patients than in sarcopenic patients. It is interesting, however, to evaluate what happens when the two conditions occur together in a patient. A work by Prado et al. aimed to ascertain the prevalence and clinical implications in patients with respiratory and gastrointestinal tract carcinomas with sarcopenic obesity(OS).

Significant differences in skeletal muscle index and a lipid lean mass were found between the sample of sarcopenic patients and the sample of non-sarcopenic patients. Muscle reduction was on average less in obese patients with sarcopenia, suggesting the presence of lipid infiltrations in the muscles.

Regarding survival, understood as the number of days of life of each patient after estimating the BMI, both univariate and multivariate analyses were conducted based on the main parameters (sex, age, type of cancer, weight changes, etc.). The most interesting data is the comparison of the survival curves between obese and obese sarcopenic patients, which confirm the importance of sarcopenia as a prognostic indicator. The study also found clues that the prevalence of sarcopenic obesity may be higher in cancer patients, both because obesity is a risk factor for some cancers and because sarcopenia predominates in elderly patients as well as for tumors. OS is a clinical and functional condition characterized by the coexistence of muscle mass and function deficit ,sarcopenia, and excess adipose tissue, characterized by a complex pathophysiology in which the two conditions negatively affect each other.

Its real prevalence is not yet fully known given the absence so far of a shared definition. A systematic review published in 2020 highlighted the extreme variability of the definitions and diagnostic approaches for OS and the profound inadequacy of the literature on the threshold values of normality and the methodological tools used (45).

During aging the modification of fat distribution often occurs, in specially it occurs in a dysfunctional adiposity typical located in visceral abdomen tissue and epicardial fat.

State of the art on automatic segmentation of body tissues through CT images

One of the most recent studies in this area is the one carried out by Borrelli et al., in which the segmentation software, based on a convolution neural network, has been trained on a training set consisting of 50 CT scans obtained from a cohort of 50 lymphoma patients, with slices 3 mm thick; the software was then tested on test sets of 148 TCs obtained from a group of 74 patients affected by prostate cancer, with slices 5 mm thick. Both sets of scans were acquired using an integrated system PET / CT. The CT images of the training set were segmented by a specialist from nuclear medicine, but limited to subcutaneous adipose tissue (SAT) and tissue muscle, thus excluding visceral adipose tissue (VAT).
They have been calculated in this way the volumes of SAT and muscle from the T11 vertebra to the caudal area of the iliac bone; voxels (volumetric equivalent of the pixel) with HU included have been identified as SAT between –190 and –30, as muscle voxels with HU between –30 and 150. For the test set manual segmentation was performed on a single slice for height acquisition of the L3 vertebra, using the same intervals of the Hounsfield scale as before. The software of automatic segmentation used (developed by RECOMIA, available online and completely free Research Consortium for Medical Image Analysis (recomia.org)) assigns to each pixel a value from 0 to 1 and the single pixel is then assigned to the category in which it obtained the highest score.
A very important point is the development of two models of linear regression, to predict the volumes of the starting tissues

from the surfaces to the L3 vertebra. For the SAT, the equation of the regression line is
y = 35.63 x + 630.3, with a linear correlation coefficient r^2= 0, 83. For the muscular fabric the model is given by the equation y = 40, 15 x + 1461, with r^2= 0, 64. All the above arguments indicate a clear convenience in using the volumes of SAT and muscle rather than L3 surfaces. Finally, one is highlighted in the study limitation of the RECOMIA software, in particular since in 9% of cases the intervention of a radiologist was necessary to correct the segmentation manually from the software itself. Another important limitation, not trivial, is that VAT was not taken into consideration, which is absolutely essential in the formulation, for example, of a therapeutic plan.
A study on a larger and more differentiated cohort was performed by Magudia, his work also aims to demonstrate the validity of automatic segmentation, carried out with a U-Net, for the calculation of body composition, always by measurements made at the level of the L3 vertebra, since these are well corrected usually related to total SAT, VAT, and skeletal muscle throughout the body.

The 3D approach to CT segmentation

All the studies reported so far assume the tissue measurements performed in the T11- L5, and especially for the L3 vertebra, as reliable for the estimation of the corporeal composition; this is due to the numerous studies that have shown in the last twenty years the correlation between 2D single slice measurements and 3D volumetric measurements. On the other hand, although 2D measurements are a good indicator for study groups, this does not imply that these

same measures must have prognostic value for individual patients. In 2004 Shen et al. have shown, in addition to the correlation between 2D and 3D measurements, that to consider several transversal slices rather than one alone provides a greater correlation. This seems to be the only way forward to increase the accuracy of the measurements, given that the regression equation found cannot be generalized to all age groups and among other categories of patients. These details and others, which in group studies may be overlooked for the effect of the large sample that is analyzed, can instead lead to errors of evaluation for individual patients; therefore it is necessary to try to conduct analyses as precisely as possible.

Obesity and cancer

Although evidence shows that adult overweight and obesity are related to risk for many cancers, the growing epidemic of obesity provides a challenge to clinical practice and the implementation of guidelines for the management of weight. Historical data from the past 25 years point to obesity as a cause of approximately 14% of cancer deaths in men and up to 20% of cancer deaths in women (KY Wolin 2010).

A major review of weight, physical activity, and cancer incidence by the International Agency for Research on Cancer (IARC) used obesity prevalence data from Europe and relative risks from a meta-analysis of published studies and concluded, in 2002, that obesity was a cause of 11% of colon cancer cases, 9% of postmenopausal breast cancer cases, 39% of endometrial cancer cases, 25% of kidney cancer cases, and 37% of esophageal cancer cases. In addition, data from the American Cancer Society suggested that overweight and

obesity were related to mortality from liver cancer, pancreatic cancer, non-Hodgkin's lymphoma, and myeloma.

Finally, emerging evidence suggests that obesity increases risk for aggressive prostate cancer. Overall, we estimate that overweight and obesity cause approximately 20% of all cancer cases. Previously Doll and Peto included overnutrition (overweight) with diet causing a combined 35% of all cancer cases.

To conclude that a cause-and-effect relation exists between obesity and cancer at each site, one often pursues studies of mechanisms that confirm the underlying biology of this relation and provide insights into prevention strategies. Take, for example, postmenopausal breast cancer. Among postmenopausal women, obesity is directly related to circulating estradiol levels, which themselves are directly related to breast cancer risk. When the action of estrogens is interrupted by estrogen receptor modulators in randomized controlled trials, breast cancer incidence is approximately 50% lower adding to the evidence of a cause-and-effect relation, a documented 50% reduction in the risk for breast cancer among women who lost ≥10 kg after menopause and kept it off adds to our understanding of this causal relation.

For colon cancer, growing evidence points to insulin pathways mediating the effect of body mass index BMI and risk. Studies of blood glucose levels and colon cancer show a direct relation between higher glucose and subsequent risk. Providing further biologic rationale, c

peptide, a marker of insulin production, also shows this positive relation, and animal models using insulin injection versus saline show a significantly higher incidence of colon cancer among those injected with insulin. Finally, preclinical

data provide additional support for the insulin–insulin-like growth factor (IGF) hypothesis of cancer risk, as outlined in several excellently detailed recent reviews

Despite extensive evidence showing a deleterious effect of overweight and obesity on cancer, relatively little data exist on the effects of weight gain or weight loss on altering the risk for cancer. The lack of data on weight loss is likely a function of the small number of individuals able to achieve a sustained weight loss.

Obesity, inflammation, and immune alterations

Obesity, as defined in adults by a body mass index greater than or equal to 30, is a growing public health problem worldwide, particularly in Western countries. The world health organization has reported that 13% of adults over the age of 18 are clinically obese, totalling more than 600 million people. In the US 34.9% of adults (age >20) are obese. It has been estimated that nearly 20% of deaths in US adults between 1986 and 2006 were related to obesity. The health risks from obesity arise from its association with the increased risk of several diseases including hypertension, type 2 diabetes, cardiovascular disease, osteoarthritis, kidney failure, liver disease and several types of cancer. The link between obesity and increased cancer incidence and cancer related deaths has been well established over the last two decades and it has been estimated that 14% of cancer deaths in men and 20% in women are attributable to obesity. As a common factor for many chronic diseases, different mechanisms have been used to explain how obesity drives their progression. Interestingly, chronic inflammation, a phenotype associated with obesity, has been known to be a major factor that

contributes to the disease progression of the above chronic conditions.

Obesity-associated inflammation is first triggered by excess nutrients, and is primarily localized in specialized metabolic tissues such as white adipose tissue, which acts as a major source of energy and is primarily composed of adipocytes. Adipocytes are endocrine cells that secrete a large range of cytokines, hormones and growth factors, referred to as adiposes, and specialize in the storage of energy as triglycerides in cytoplasmic lipid droplets. Excess nutrients leads to activation of metabolic signalling pathways including c-Jun N-terminal kinase (JNK), nuclear factor κ B (NFκB), and protein kinase R. Activation of these pathways leads to an induction of low-level of inflammatory cytokines resulting in a low-grade inflammatory response. Excess nutrients and obesity also lead to the hyperplasia and hypertrophy of white adipose tissue adipocytes, as well as to the extensive tissue remodelling and an increase in free fatty acids resulting in changes in adipokine production and a low-grade inflammatory response. Obesity also leads to increased endoplasmic reticulum stress resulting in activation of the unfolded protein response, which leads to activation of NFκB, JNK and increased oxidative stress, and in turn the up-regulation of inflammatory cytokines. All these pathways contribute to the initiation of obesity associated inflammation. While obesity associated inflammation is primarily localized in white adipose tissue, other tissues have been shown to have increased inflammation because of obesity, including the liver, pancreas, and brain.

This low-grade inflammatory response associated with obesity leads to changes in immune cell infiltration and polarization in white adipose tissue. In particular, macrophages are the major innate immune cells that are recruited to white adipose tissue under obesity and are one of the major sources of inflammatory cytokines in obese white adipose tissue. The recruitment of macrophages into obese white adipose tissue is mediated by a few different mechanisms, 1) adipose tissue macrophages and adipocytes secrete a milieu of elevated levels of chemokines, including CCL2, CCL3 and RANTES/CCL5, which promote the recruitment of macrophages into obese white adipose tissue; 2) obesity-induced adipocyte hypertrophy leads to increased adipocyte cell death, in turn recruiting macrophages to phagocytize the dead adipocytes; 3) adipocyte hypertrophy and cell death leads to increased levels of free fatty acids (FFAs), which act as TLR4 agonists and likely ligands for nod-like receptors to induce an inflammatory response and recruitment of macrophages in white adipose tissue. These mechanisms work in concert to induce a large increase in adipose tissue macrophages in obese white adipose tissue.

In addition to increase adipose tissue macrophages in white adipose tissue from obese individuals, white adipose tissue can also shift the polarization of macrophages, from an anti-inflammatory M2-like phenotype in lean white adipose tissue, to a more pro-inflammatory M1-like phenotype in obese white adipose tissue. This is partly due to the imbalance of obesity-related adipokines, i.e. the change in leptin and adiponectin ratio. Obese white adipose tissue tends to have an increase in the production of leptin, which is pro-inflammatory, proangiogenic, and proliferative, and a

decrease in adiponectin, which is anti-inflammatory, antiangiogenic and anti-proliferative. The increased leptin level leads to monocyte differentiation into macrophages and repolarization of adipose tissue macrophages. Recent studies have suggested that adipose tissue macrophages in obese white adipose tissue may not be classically activating M1 macrophages. Using proteomic and other techniques, Kratz et al. has recently shown that adipose tissue macrophages from obese humans and animals have a distinct phenotype that express inflammatory cytokines associated with M1 macrophages but lack other characteristics of M1 macrophages. The pro-inflammatory adipose tissue macrophages in obese white adipose tissue recruit other immune cells, and along with adipocytes secrete more than 50 different cytokines, hormones, and chemokines, all of which contributing to the chronic inflammation associated with obesity.

The impact of obesity on immunity is not limited to macrophages. Recent literature has identified a panel of immune alterations, including those from both adaptive and innate immunity, that are impacted by obesity, including the increased Th1 cell response, CD8 cytotoxic T cell response, natural killer (NK) cells etc. as well as the decreased number of regulatory T cells. The interaction between these cells in adipose tissues is very complex. For example, CD8 T cells have been shown to be the early event showing up in adipose tissue of diet induced obesity, which plays an important role in further recruiting M1 macrophages and subsequent inflammation. Th1 cytokines are also the known activator of M1 macrophages in general and this axis has been established

in adipose tissue of obese individual and contributes to insulin resistance.

Among all the immune alterations, obesity-associated inflammation is of particular interest because the pathophysiology of many of the major human diseases associated with obesity, including type 2 diabetes, cardiovascular disease, and cancer, have been linked to inflammation. Here we would try to discuss the effect of obesity on, and the role of obesity-associated inflammation in the carcinogenesis and disease progression of types of cancer such as colon cancer.

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Body Composition: adipose tissue and the differences in men and women

The body composition between man and woman is often underestimated both in terms of function and deposition of adipose tissue; the compartments of adipose tissue vary significantly according to both sex and age. Women compared to men differ as regards to the distribution of adipose tissue, they need an overall content of total body fat higher than men, especially in the fertile period; the adipose tissue plays a pivotal role in the homogenises, and the subcutaneous adipose tissue is, in fact, the seat of receptors suitable to product estrogens.

Women accumulate more fat in the subcutaneous deposit before menopause and this is a feature associated with protection from the negative consequences associated with obesity and metabolic syndrome. The SAT, in fact, is a body considered protective with respect to the VAT. After menopause, fat deposition and accumulation changes, favouring a visceral deposit. This hormonal change has

been demonstrated in functional studies on cancer patients who used aromatase inhibitors for breast cancer. Over the years these patients developed a typically apple-like body shape like men in relation to secondary aldosteronism possibly related to therapy (i.e. aromatase inhibitor Battisti 2014).The increase in VAT in postmenopausal women is accompanied by a parallel increase in metabolic risk very similar to that seen in men. The latter, in fact, tend to accumulate more visceral fat, leading to the classic apple shape, also called android, which has been highly correlated with the increase in cardiovascular risk.

About 80% of all body fat is found in the subcutaneous deposit and is found just under the skin mainly around the waist, in the subscapular area and in the gluteal and hamstring areas (thigh). Visceral fat, which represents 10-20% of total fat, is found in the abdomen mainly in the omentum and mesentery, but also in perirenal, gonadal, epicardial and retroperitoneal deposits. Visceral fat accounts for a higher percentage of total fat in men than in women.

Obesity is a condition influenced by a number of variables such as ethnicity, socio-economic status and education that makes it difficult in humans to determine if there is a biological difference per se in the propensity to gain weight among men and women. On the contrary, in animal models in which non-biological factors are excluded, studies suggest that the propensity to develop obesity differs between the sexes and this is directly due to sex hormones. For example, female rats gain less weight than males when presented with a metabolic challenge such as a high-fat diet, a difference that is no longer seen after ovariectomy (Stubbins, et al. 2012). This is because estrogens protects against increased body

adiposity/obesity through their effects to suppress appetite and increase energy expenditure. Many postmenopausal women gain body weight due to the natural decrease in endogenous estradiol levels but the reduction in energy expenditure can be prevented with estrogen replacement therapy (Gambacciani et al. 1997). These observations, therefore, show that estrogen suppresses food intake and increases energy expenditure in women. Pre-menopausal women tend to store fat on the hips, thighs and buttocks, giving them a pear shape also called a gynoid, or gluteal femoral pattern of fat distribution. Men accumulate fat predominantly in the abdominal region giving them an apple shape, also known as an android, or abdominal fat storage pattern. The differences in the distribution of adipose tissue are related to the specific differences of the adipose deposit in the absorption and storage of fat.

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The differences in distribution of adipose tissue are related to specific differences of the adipose deposit in the absorption and storage of fat. The limiting phase of the accumulation of fat derived from circulating fatty acids and triglycerides is the activity of the lipoprotein lipase. The activity of this enzyme is higher in the buttock (subcutaneous) than in abdominal (visceral) fat in women, facilitating their gynoid distribution. On the contrary, the enzymatic activity is higher

in abdominal / visceral adipose tissues in men (Arner et al. 1991).

These sexual differences in distribution of adipose tissue are accentuated through a suppressive effect of testosterone on activity of lipoprotein lipase in subcutaneous femoral fat in men (Ramirez et al. 1997). Visceral adipose tissue has been identified as a risk factor for colorectal cancer (CRC), more accurate than waist circumference, regardless of body mass index.

The estimated attributable personal risk of colon cancer is 10.92% in men and 2.57% in women, and 5.05% for rectal cancer in men (Renehan AG, et al. 2010)

The incidence of colon cancer is significantly higher in men with obesity, but waist circumference and the increase in the waist-hip ratio have also been associated with significant increases in CRC. Instead, in women, the association between obesity and colon cancer is weaker.

In summary, BMI appears to be associated with an increased risk of colon cancer in both genders, but less so in women. This gender difference could be explained by the protective effect of oestrogen attributable to the induction of apoptosis and the inhibition of cell proliferation (Chen J. et al. 2012).

Women have a higher risk of developing right (proximal) colon cancer than men, which is associated with a more aggressive form of cancer than left (distal) colon cancer (Hansen IO et al. 2012). In advanced colon cancer, proximal colon cancers are more often flat, while distal colon cancers are polypoid, which is more distinguishable from colonoscopy (Kaku E, et al. 2011). Furthermore, women have a longer transverse colon than men who have a lower detection rate on colonoscopy (Saunders BP, et al. 1996).

Hormonal factors may explain the large percentage of right colorectal cancer in females. A population-based case-control study examining sex, reproductive factors and hormone exposure related to microsatellite instability (MSI) in colon cancer suggested that oestrogen exposure is a protective factor against cancer, while the lack of oestrogen in older women increases the risk of colon cancer. In the same study, hormone replacement therapy (HRT) was associated with a reduced risk of unstable tumours (Slattery ML, et al. 2001).
The Women's Health Initiative Clinical Trial reported that postmenopausal women undergoing hormone replacement therapy showed a 40% reduction in colorectal cancer risk, while women undergoing hormone replacement therapy while being diagnosed with cancer colorectal cancer, showed a higher grade/stage of colorectal cancer (Reitenbaugh C, et al. 2008). These results indicate that hormone replacement therapy could have a detrimental effect on colorectal cancer risk after cancer development. Additionally, women over the age of 65 show higher mortality (Ferlay J SI, et al. 2013) and a lower 5-year survival rate of colorectal cancer than their age-matched male counterparts (Park HC, et al. 2013).

CT SEGMENTATION

Segmenting an image means recognizing elements with common characteristics within it, distinguishing these elements from others with different characteristics and grouping all similar elements into regions, outlining borders between them.

In a digital image, segmentation consists in somehow classifying and quantifying the properties of each pixel, such as intensity for monochrome images, colour and texture.
Manual segmentation of body tissues is a repetitive, time-consuming and operator-dependent process; consequently, it is not carried out regularly on all CT images acquired by patients, although doing so would lead to numerous advantages in several areas.
In many structures, however, the segmentation of body tissues is also performed by means of semi-automatic segmentation software: these are software that propose a segmentation of the image, which however needs to be checked and corrected manually by a radiologist.
Returning to the theoretical basis of segmentation, the simplest method is "threshold", based on the assumption that different regions of an image are characterized by different intensity values. This method is based on finding a grey threshold value such that if a pixel exceeds that value, it is considered an object pixel (foreground), while if it does not exceed the threshold it is classified as a background pixel.
In addition to thresholding, segmentation algorithms for monochrome images work based on one of the following two properties: discontinuity and similarity. The most used approach for the first category is edge-based segmentation, which in order to work well requires that the edges of the regions are sufficiently different from each other and from the background, in order to allow recognition of edges based on local intensity discontinuities. The second category, on the other hand, exploits the region-based segmentation, which consists in collecting in a group, the cluster, pixels with sufficiently similar properties to form together a

homogeneous region; the homogeneity criterion is usually determined by the grey level of the pixels. Recent automatic segmentation software is based on deep learning, a branch of machine learning based on neural networks of algorithms and which deals with self-learning methods for carrying out complex tasks. This is possible thanks to the automatic creation by the machine of hierarchical statistical models, built starting from the simplest information to arrive at the most complex representations. Each piece of information contained in a representation is called a feature; features created by a human and supplied to the machine are called hand crafted features. More precisely, a feature is an individual and measurable property of an observed phenomenon, usually rendered in numerical form. An important feature of deep learning networks is that they consist of a series of successive layers: at each layer, the input signal is processed and passed to the next layer. The layers that are located between the input and the output are defined as hidden and a network that has this structure is called a neural network.

TEST 2:

Materials: Use AI pre-trained machine learned software DAFS v3.0.2 (VORONOI Inc)

Methods: random selected patients in double blind 12 patient. Conclusion: software in the trial version was perfect, very powerful, only 120 minutes for a 3D analysis of all whole body analysis segmentation ,but human time (i.e. interaction with computer-human).

still needed too much time. Consequently, the institution prefers not to buy the software at the moment.

TEST 3:

Objective: are modifications in the human body visible on CT in healthy? Can we find an indicator as VAT/SAT?

GROUP: Patients' healthy females were retrospectively identified in the PACS database of institution.

Subjects underwent a CT scanner, in a supine position in basal condition, in emergency department for acute abdominal pain caused by kidney calculi. The patients were grouped by menopausal status. The patient's selection criteria were exclusion of mental disease such as Alzheimer, or any disease or past history correlated with alimentary disorder, chronic inflammatory disease or vertebral fractures.

CT measurements of fat tissue were assessed using tree-millimetre slices images which were acquired covering all abdomen volume. Abdominal adipose tissue measurements were quantified semi automatically on a dedicated workstation (Osirix).

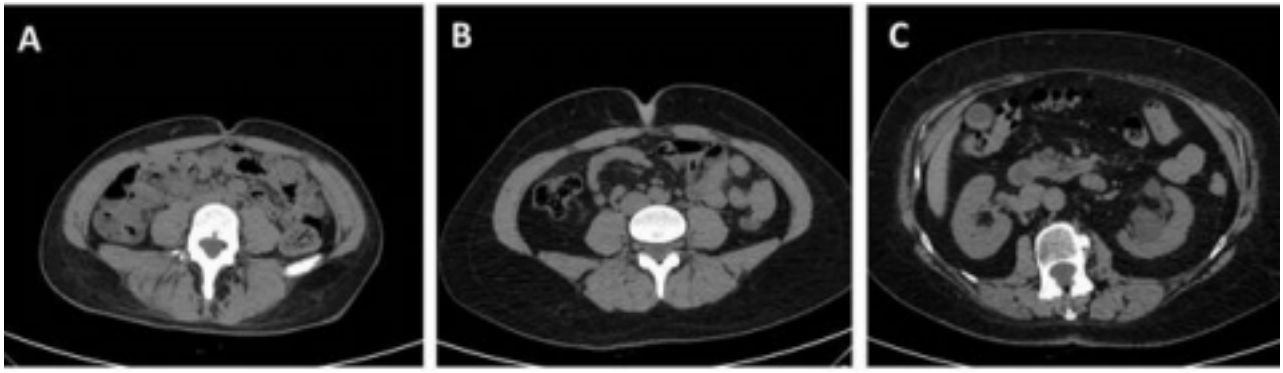


Figure 2) CT axial images acquired at emergency department in basal condition in patient at age 28yrs (A) 46yrs (B) 71yrs (C) showing an increas of total adipose tissue (TAAT) in particular visceral adipose tissue (VAT) rises among older individuals.

The TAAT was automatically generated by the workstation, while the SAT was traced manually, considered as a limit to the muscular wall, to separate VAT from the SAT deposit. The VAT values were the result from the formula TAAT-SAT. Bone density was calculated positioning a region of interest

(ROI) in cortical and trabecular L1 body to measure Hounsfield unit (HU), the percentage of the trabecular/cortical ratio (T/C index) was calculated.

Reproducibility

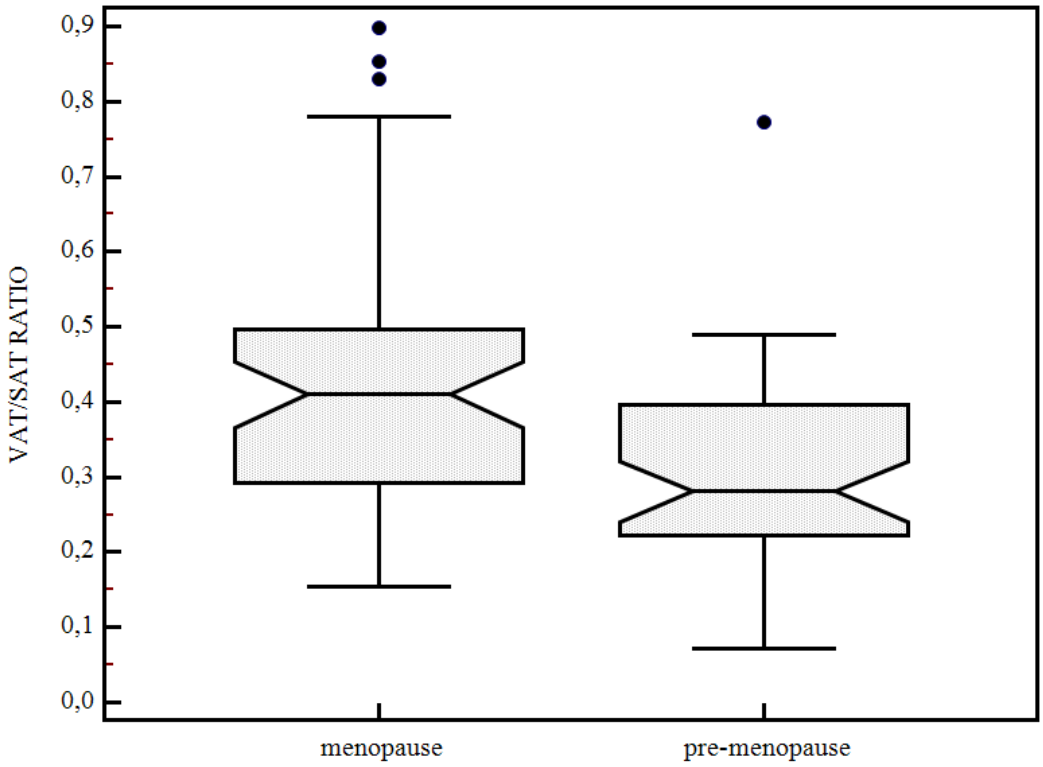
Intra-reader reproducibility was analysed by redefining ROI and re measuring values in 10 patients at least 1 week later than the first reading. Linear correlation was performed to assess age and TAAT, VAT, SAT and T/C index.

Paired T-student test was used to compare VAT/ SAT before and after menopause status.

Results

K Cohen was for inter-rater reliability 0.95, while intra-rater reliability was 0.97. Value of TAAT, VAT e SAT increase with age before menopause TAAT 8325,2± 4228,3$mm^3$to after 12652,9± 5122,8mm^3 Modification of VAT/SAT ratio was observed in all subjects, reflecting a relatively increased volume of VAT with a reduction or stability of SAT with ageing. Median VAT/SAT ratio in post-menopause was 0.41 (CI 0.36 to 0.45; lower value 0.15 highest 2.5); in pre-menopause was 0.28 (CI 0.24 to 0.34; lower value 0.07 highest 0.77); difference between the two groups was significant using t- test (p=0.0004; graph 2).

Graph 2



The ratio L1 T/C index showed a decreasing value ageing linearly (r=- 0.61) 95% CI from -0.72 to 0.47 significance level P<0,0001 (Table 2 , Graph 3)

Table 2.

Hormonal status														
	After Menopause							Before Menopasue						
	N	Mean	95% CI	SD	Median	Minimum	Maximum	N	Mean	95% CI	SD	Median	Minimum	Maximum
TAAT	54	12652,9	11254-14051	5122,8	11650,5	3530,0	26262,0	46	8325,2	7069 - 9580,9	4228,3	7508,0	2555,0	20209,0
VAT	54	3698,2	3202,0 - 4194,5	1818,1	3421,0	885,0	8569,0	46	1903,6	1513,3 - 2293,8	1314,1	1318,000	565,0	5513,0
SAT	54	8954,7	7955,8-9953,5	3659,4	8500,5	2433,0	18152,0	46	6428,78	5499,0-7358,5	3130,8	6014,0	1441,0	15902,0

Graph 3

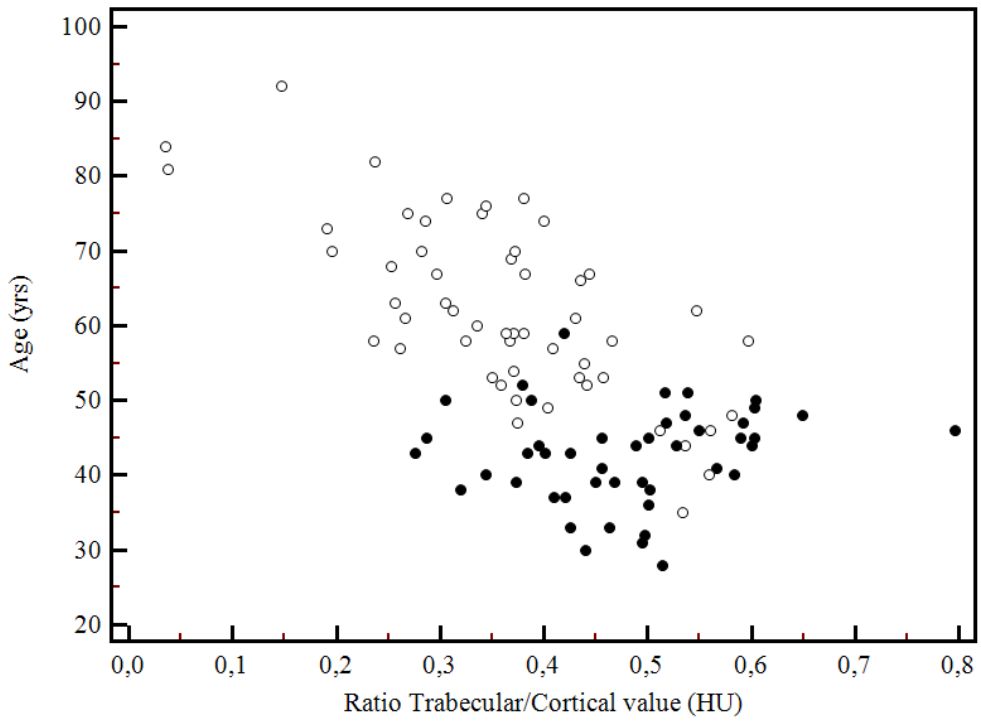


Table 3

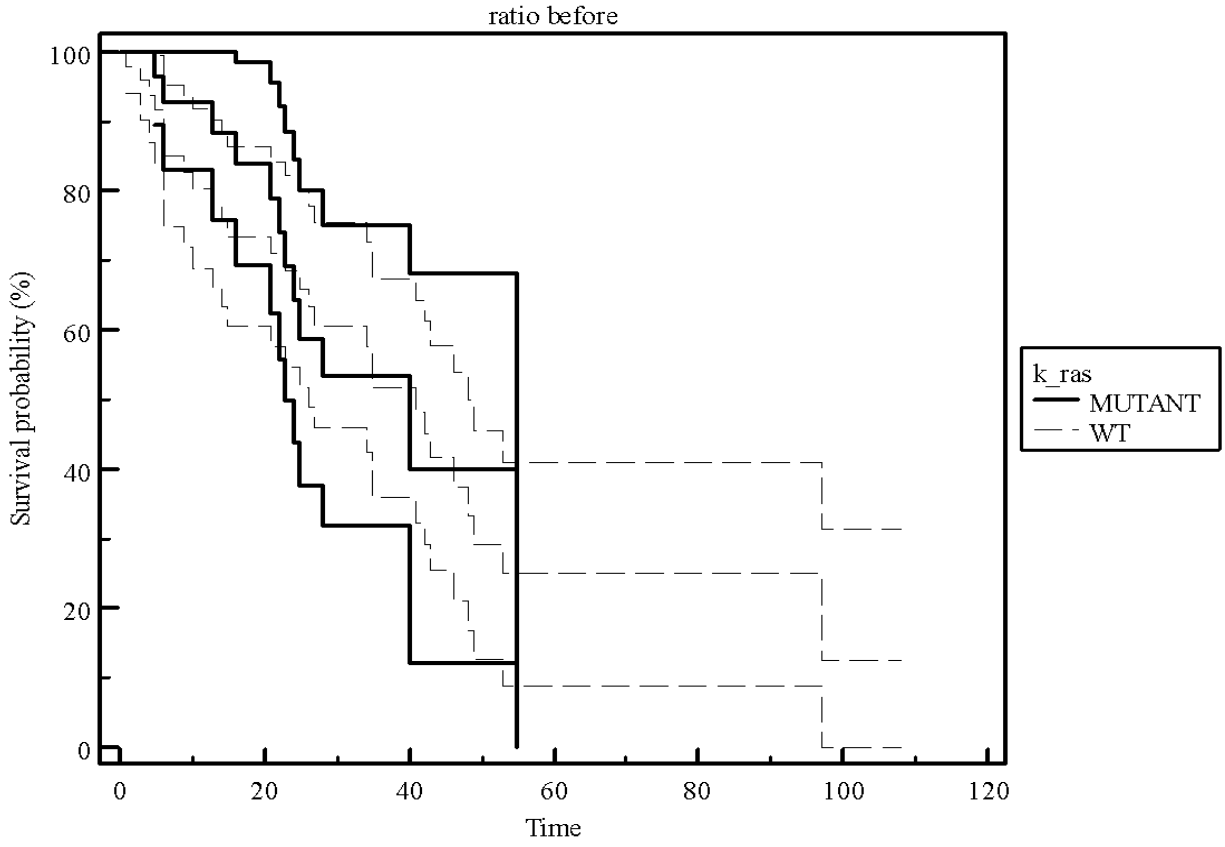
Hormonal status

	After menopause							Before						
	N	Mean	95% CI	SD	Median	Minimum	Maximum	N	Mean	95% CI	SD	Median	Minimum	Maximum
L1_trabecular_bone	54	155,0	142,2 - 167,8	46,92	145,0	66,0	280,0	46	203,1	189,0 - 217,2	47,37	205,5	78,0	320,0
L1__cortex	54	428,3	405,2 - 451,4	84,72	433,0	247,0	667,0	46	430,4	404,3 - 456,6	88,08	416,5	254,0	757,0
ratio_TC	54	0,36	0,32-0,39	0,12	0,37	0,035	0,597	46	0,47	0,44 - 0,51	0,10	0,492	0,276	0,796

Conclusion: ageing is accompanied by a change in the distribution of body fat with increased VAT/SAT ratio after menopause, in addition, a decreasing value of the T/C index was observed based on the osteoporotic alteration. CT is a useful tool for describing the composition of body fat in all patients.

TEST 4

This study aims to highlight the role of visceral adipose tissue in metastatic colon cancer during time; this is a cohort of patient followed for a total of 10 years, to try to define value of VAT/SAT. It is a retrospective part of the study.



Graph 4

The data used were taken from venous phase CT scans from patients with metastatic colon cancer treated by chemo-immunotherapy with Cetuximab. Colorectal cancer (CRC) is one of the most common malignancies in the world. There are 150,000 cases in Europe. They currently account for 15% of all cancers and are an important cause of cancer mortality in men, after lung cancer, and in women, after breast cancer. This neoplasm is rare before the age of 40, but occurs more frequently around the age of 60.

Despite the increase in the incidence observed in recent years, a simultaneous reduction in mortality has been recorded, mainly due to the greater ability to identify the neoplasm in increasingly early stages, and to the improvement in the effectiveness of therapeutic treatment.

Its aetiology is unknown, although dietary habits (low-fibre and high-fat diet), genetic factors, inflammatory bowel diseases, a sedentary lifestyle and obesity are considered among the risk factors. To date obesity, and in particular visceral fat, in patients with metastatic colon cancer, does not have a fully established role, but recently it has been shown that the latter is able to influence the outcome of the disease.
Several studies have shown a strong association between obesity and the risk of developing major colon cancer in men rather than women, as well as a high rate of cancer recurrence and mortality in obese patients compared to normal weight.
Abdominal fat as a pathological marker of disease is being studied, as well as its role in survival and quality of life.
This thesis aims to highlight the role of total abdominal adipose tissue (TAAT) and its distributions in patients with advanced colon cancer. Abdominal fat is divided into two compartments: subcutaneous (SAT) and visceral fat (VAT), where the latter is associated with the increase
in the incidence and recurrence of colon cancer.
The first objective of this study is to retrospectively assess whether there are any changes in the distribution of adipose tissue before and after Cetuximab-based chemotherapy.
Furthermore, the second objective is to evaluate PFS (progression-free survival), that is the period of time in which a person with cancer continues to have the disease, without worsening it.

Materials and methods

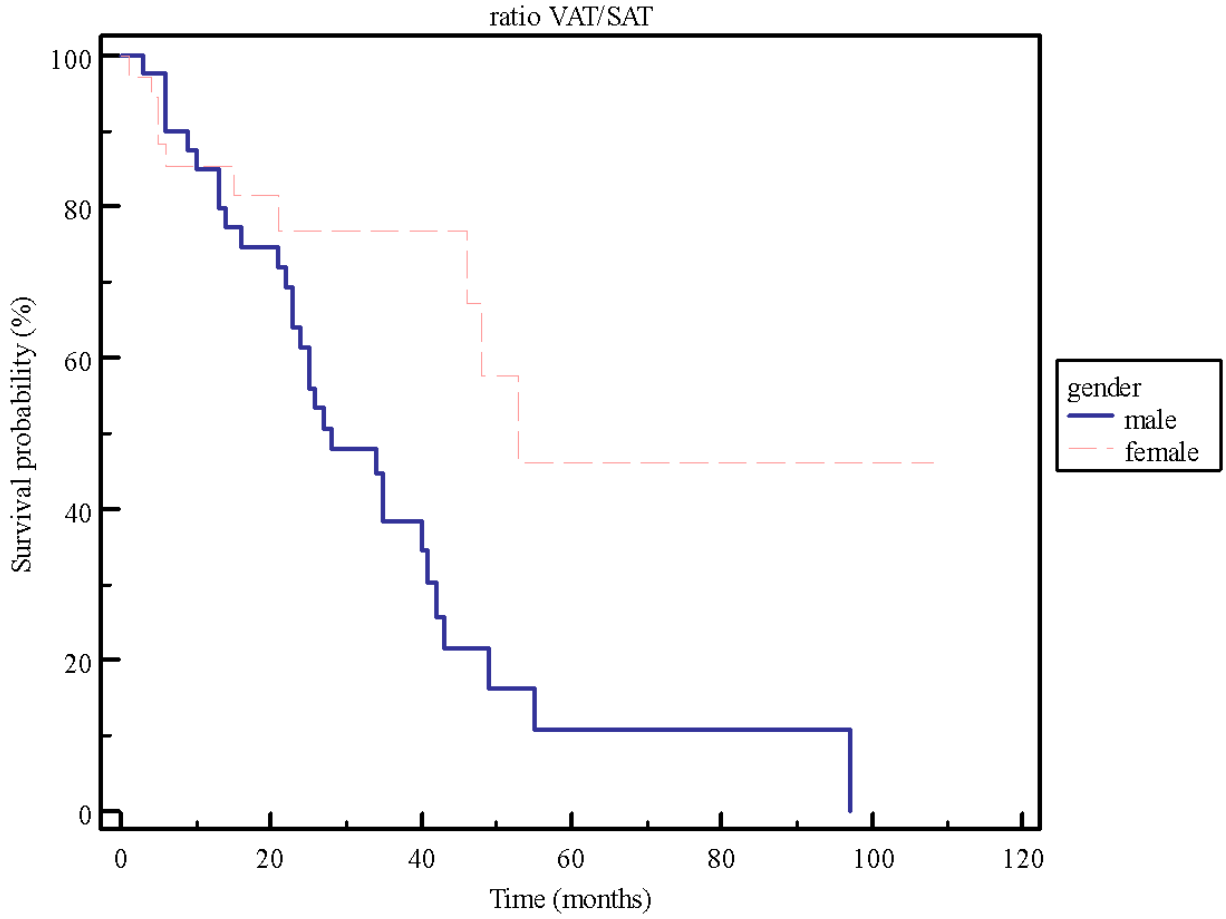
We conducted a single-centre retrospective observational study on metastatic colon cancer patients who followed a Cetuximab therapy protocol. The study carried out is aimed

at evaluating the effects that Cetuximab therapy causes on patients with metastatic colon cancer (mCRC). K-RAS mutation status had to be verified prior to initiating treatment with cetuximab.

The K-RAS test allows to determine, at the time of diagnosis, the status of the gene that codes for the K-RAS protein, whether normal or mutated, in a patient's colorectal cancer. The test, therefore, identifies an oncological biomarker, or the K-RAS protein, which can predict the response of colorectal cancer to personalized therapies.

Cetuximab should not be used in the treatment of patients with colorectal cancer with the K-RAS mutation, as a negative benefit-risk ratio has been shown. The patients were then subjected to a computed tomography (CT) examination with clinical scanners performed before and after the initiation of cetuximab therapy.

Graph5



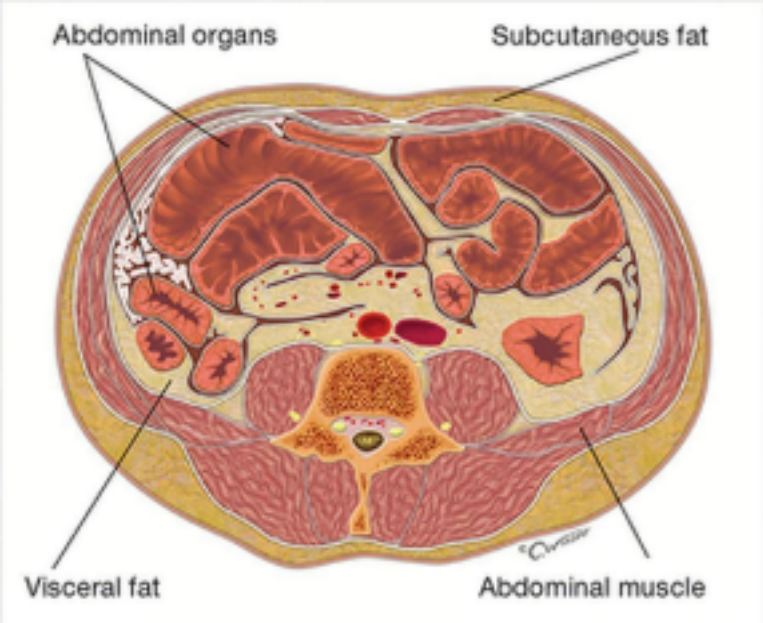
The measurements of adipose tissue by CT were performed using the images of the complete abdomen acquired during the venous phase with a thickness of 3 millimetres.

More specifically, changes in total abdominal fat (TAAT), subcutaneous abdominal fat (SAT) and visceral abdominal fat (VAT) distribution were observed.

In order to determine the volumes of the adipose tissue, two workstations were used: Leonardo and Osirix. The Osirix workstation automatically generated the TAAT, while the SAT was generated by means of a semi-manual tracking, using the external surface of the abdominal muscles as a reference point. Instead, the VAT was calculated as the result of the subtraction between TAAT and SAT.

The data collection was based on 3 main steps:
- Calculation of the TAAT
- Calculation of the SAT
- Calculation of VAT

Fig 3



Distribution of total abdominal fat (TAAT), subcutaneous abdominal fat (SAT) and visceral abdominal fat (VAT).

Calculation of the TAAT

The Osirix software was used to calculate the TAAT (total abdominal adipose tissue). Once the images of the venous phase have been viewed, since it is not included in our study, the chest is isolated. We therefore position ourselves on an axial scan on which the region of interest will be set. Once the area of interest has been marked on the entire CT scan, the volume in cm^3 must be calculated.

Calculation of the SAT

Subsequently, after the evaluation of the TAAT, the SAT (subcutaneous adipose tissue) is evaluated. To calculate

the SAT, the "Leonardo" machine was used, with which the volume of interest was contoured by taking the abdominal wall muscles as a reference, eliminating its content, and by reconstructing the images the SAT was obtained. The last step consists in calculating the volume in cm^3 of the contoured subcutaneous adipose tissue. To do this, the image obtained with the "Leonardo" machine must be saved and transferred to the Osirix program, with which the same procedure used for calculating the TAAT will be carried out.

Calculation of VAT

Once the values in cm^3 of the TAAT and SAT volumes have been obtained, the calculation of the VAT (visceral adipose tissue) derives from the subtraction of the two volumes obtained. By subtracting the volume of the subcutaneous adipose tissue from the volume of the abdominal adipose tissue, the volume of the visceral adipose tissue is obtained.

Statistical analysis

The primary endpoint is progression free survival (PFS) calculated from the date of the CT scan until the last follow-up, death or tumour progression.

The qualitative variables were expressed in frequencies and percentages were evaluated using the statistical Chi-square test. Statistical analyses were performed with SPSS 20 (IBM SPSS Statistics for Windows, version 20.0, IBM Corp, Armonk, NY).

Results

Clinical characteristics of the population studied

88 subjects were examined including 53 males and 35 females with an average age of 65 years, an average weight and height

of 70 kg and 168 cm and an average BMI of 25.13 (overweight) (Table 6 and 7).

Table 4. *Gender male/female*

		Frequency	Percentage
Gender	0 (M)	53	60,2
	1 (F)	35	39,8
	Total	88	100,0

Table 5. Age, BMI, height/weight

	Age (yrs)	BMI (kg/m²)	Peso	Altezza
Median	65,4114	25,129807	70,00	168,00
Minimum Value	33,90	17,9012	42	149
Maximum Value	86,95	36,8980	113	187

We then checked how many patients have a mutational status of the KRAS gene. Evaluation of KRAS mutational status is important from the time cetuximab therapy is initiated as it has been seen to represent a mechanism of resistance to this.

The statistical analysis revealed that of these 88 patients only 37 had mutated KRAS, while the remaining 51 patients were wild type (Table 6).

Table 6. *K-RAS*

		Frequency	Percentage
K-RAS	Wild type	51	58,0
	mutant	37	42,0
	Total	88	100,0

Body fat distribution in the population studied

As already mentioned above, this study aims to highlight the role of total adipose tissue (TAAT) and its distributions, understood as subcutaneous abdominal fat (SAT) and visceral

abdominal fat (VAT), in patients with advanced colon cancer before and after cetuximab therapy.

Table 7. TAAT, SAT and VAT measurements before and after cetuximab treatment in both sexes.

		TAAT before (mm^2)	TAAT After (mm^2)	SAT before (mm^2)	SAT after (mm^2)	VAT before (mm^2)	VAT after (mm^2)	VAT/ SAT before (mm^2)	VAT/SAT after (mm^2)
Median		9721,00	10494,50	4671,50	4704,00	5202,50	5473,50	1,144728	1,343530
Minimum Value		3360	3042	207	406	710	833	,0960	,1296
Maximum Value		22494	21830	12051	10883	15508	14263	15,2319	9,9236
Percentile	25	7630,00	8084,75	2794,75	2863,50	3393,75	4120,50	,765607	,928285
	50	9721,00	10494,50	4671,50	4704,00	5202,50	5473,50	1,144728	1,343530
	75	13077,50	12779,25	6886,50	5998,25	6882,75	7373,50	1,704027	1,954529

From tables 7, 8 and 9 we can see that the subcutaneous adipose tissue (SAT) is greater in women than in men; on the contrary, visceral adipose tissue (VAT) is higher in men than in women. Women compared to men differ in the distribution of adipose tissue, they need an overall content of total body fat higher than men, especially in the fertile period.

Women, having a high level of oestrogen after puberty, have a higher percentage of body fat than men and tend to accumulate more SAT, particularly on the hips, thighs and buttocks (gynoid, "pear" distribution), while men accumulate more VAT (android, “apple” distribution).

Furthermore, from these tables we can also see the changes in adipose tissue distribution before and after cetuximab chemotherapy. What emerges is that patients with metastatic colon cancer have an increase in both TAAT, SAT and VAT

after treatment with cetuximab, as well as an increase in the VAT / SAT ratio.

Table 8. *TAAT, SAT and VAT measurements before and after cetuximab treatment in males.*

		TAAT before (mm^3)	TAAT after (mm^3)	SAT before (mm^3)	SAT after (mm^3)	VAT before (mm^3)	VAT after (mm^3)	VAT/ SAT before (mm^3)	VAT/ SAT after (mm^3)
Median		9346,00	10010,00	3590,00	3932,00	5601,00	6388,00	1,254572	1,535088
Minimum Value		3360	3485	207	406	1593	1633	,3362	,8254
Maximum Value		22229	21830	12051	10883	11584	12855	15,2319	9,9236
Percentile	25	7474,50	7975,50	2472,00	2517,00	3515,00	4139,50	1,022136	1,001094
	50	9346,00	10010,00	3590,00	3932,00	5601,00	6388,00	1,254572	1,535088
	75	11914,50	12211,50	5133,00	5284,50	7077,00	8029,00	1,861677	2,213446

Table 9. *TAAT, SAT and VAT measurements before and after cetuximab treatment in females.*

		TAAT before (mm^3)	*TAAT after (mm^3)*	*SAT before (mm^3)*	*SAT after (mm^3)*	*VAT before (mm^3)*	*VAT after (mm^3)*	*VAT/ SAT before (mm^3)*	*VAT/SAT After (mm^3)*
Median		10626,00	11480,00	6521,00	5765,00	4080,00	5023,00	,770232	,939410
Minimum Value		4931	3042	2372	1408	710	833	,0960	,1296
Maximum Value		22494	20978	10163	10101	15508	14263	2,3433	2,6685
Percentile	25	8107,00	8156,00	4620,00	4372,00	3364,00	3750,00	,612776	,682343
	50	10626,00	11480,00	6521,00	5765,00	4080,00	5023,00	,770232	,939410
	75	14319,00	12945,00	7629,00	7469,00	6413,00	6265,00	1,077973	1,399316

Association between PFS, obesity and body fat distribution

In addition, the findings show that overweight and obese patients have better progression-free survival (PFS) than normal-weight patients. Progression-free survival is the period of time in which a person with cancer continues to have the disease, without it getting worse. It is used as an index of treatment effectiveness and can be an important goal in the management of very aggressive cancers that normally progress despite treatment. Furthermore, PFS is not only an indication of prolonged survival, but also of a better quality of life for the patient with cancer.

It is important to emphasize that the protective effect of obesity should only be understood as a greater efficacy of the anti-EGFR therapy in overweight and obese patients than in normal weight or underweight patients, as the latter are more sensitive to the toxicity of the therapy.

This is because the patient of normal weight tends to have a greater volume of distribution of chemotherapy drug than the patient with a higher BMI.

Therefore, on the one hand it can have greater efficacy on the tumour, but on the other it exposes the patient to a greater risk of having drug induced toxicity, this is because the compartments of fat and lean tissues represent the distribution sites of lipid and water-soluble drugs, respectively.

This concept can be expressed as the term "obesity paradox", which was coined to indicate the apparent best prognosis in

overweight or obese cancer patients, revealing a positive correlation between BMI and progression-free survival.

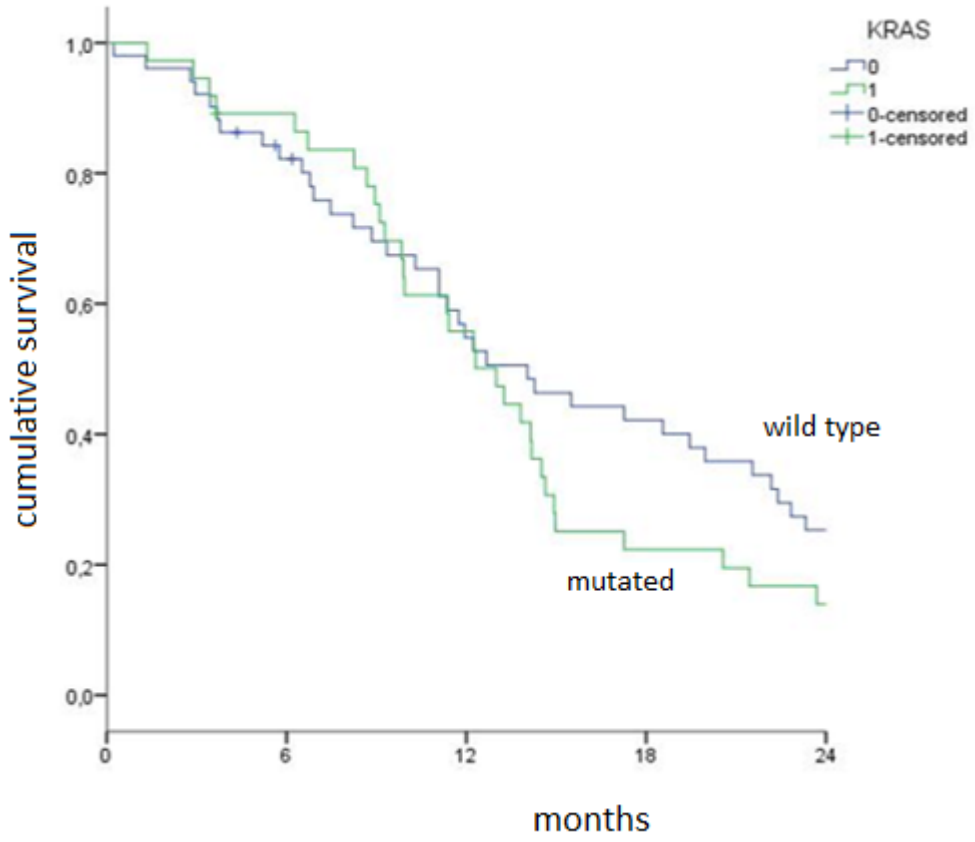
PFS 12-month value for all calculations

PFS according to KRAS. It does not reach significance for the bounded n. But there is a mutated KRAS trend that is worse even if it is not significant in this cohort (55 ± 7% vs 55 ± 8%; p = 0.11).

Table 10. PFS 12-month value for all calculations

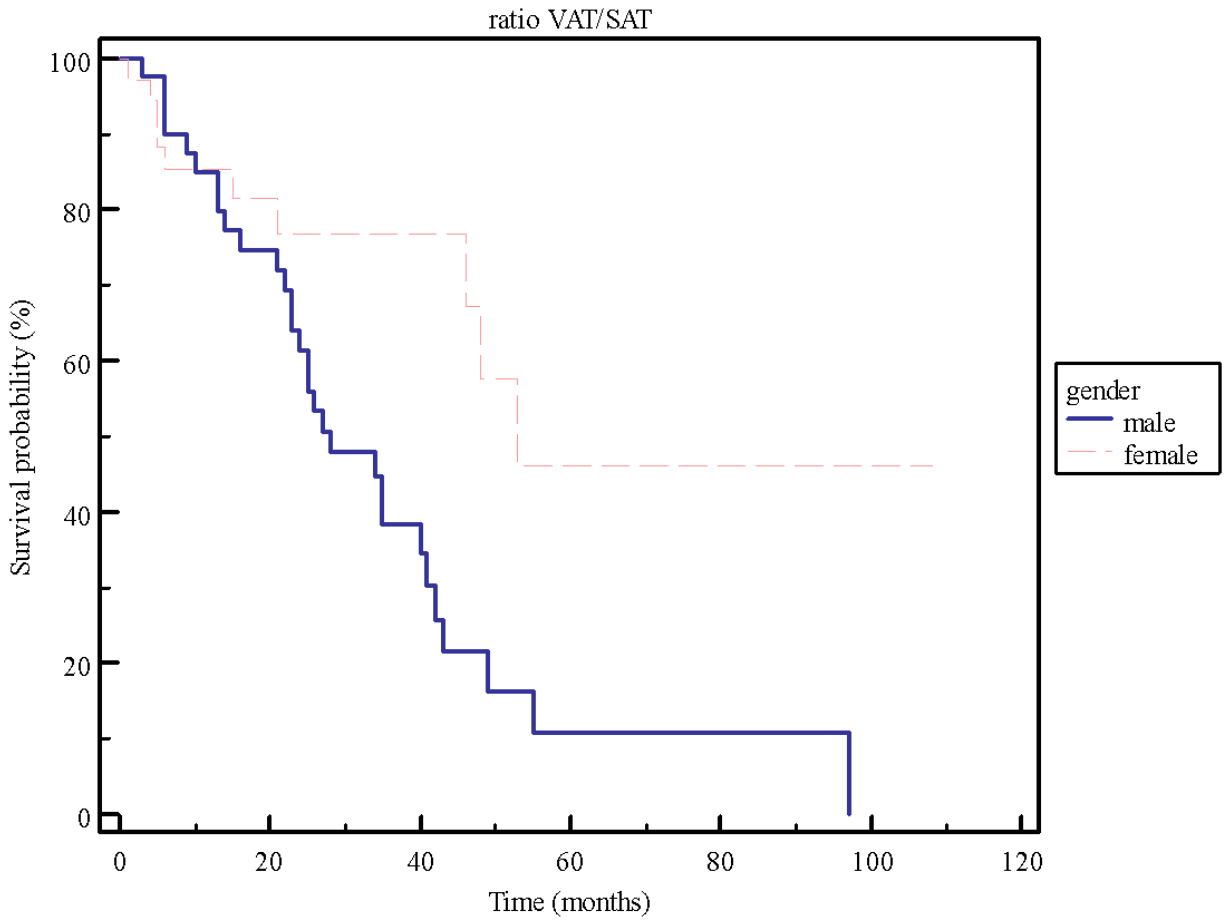
PFS on accordance to the TAAT median	< median	45 ± 8%
	> median	66 ± 7%
	P	0.06
PFS on accordance to the SAT median	< median	50 ± 8%
	> median	60 ± 7%
	P	0.06
PFS on accordance to the VAT median	< median	39 ± 8%
	> median	72 ± 7%
	P	0.12
PFS on accordance to the VAT/SAT median	< median	53 ± 8%
	> median	58 ± 7%
	P	0.90
PFS on accordance to overweight/obese vs normal-underweight	> 25	52 ± 9%
	< 25	74 ± 7%
	P	0.03

Graph 5. Kaplan-Meier (MK) estimator



Graph 5 represents the Kaplan-Meier (KM) estimator, also known as the limit product estimator. It is used to estimate the survival function of life span data. As we can see from the graph, the 12-month survival of both patients with mutated and wild-type KRAS does not differ significantly, but after 18-24 months the two curves separate showing that patients with mutated KRAS have a survival less than in patients with wild type KRAS.

Graph 6



Graph 6: This KM tried to follow the same group of patient after last CT acquired in PACS archive during 2015 ; it demonstrate that there is a important gender difference in female patients

Comparison of survival curves (Logrank test)

Chi-squared	7,7347
DF	1
Significance	P = 0,0054

Conclusions

In summary, changes in adipose tissue distribution, understood as SAT and VAT, before and after cetuximab therapy were observed in this study, and an increase in visceral adipose tissue (VAT) emerged in treated metastatic colon cancer patients with cetuximab therapy. Unfortunately it is not a clinical indicator of disease but probably the cause is linked to the lower number of patient.

In addition, progression-free survival (PFS) was evaluated and the statistical analysis showed that this is better in obese and overweight patients than in normal weight patients.
The role of obesity and in particular of visceral fat in patients with metastatic colon cancer is not fully understood to date.
VAT has been associated with an increase in the incidence of colon cancer and its recurrence, as well as negatively affecting the survival of these patients.
Among the deleterious effects of VAT there is an association with chronic inflammation and an increase in circulating pro-inflammatory cytokines, which are involved in a series of diseases related to obesity, such as hypertension, inflammation, atherosclerosis, dyslipidemia, type 2 diabetes, metabolic syndrome, oncological diseases, etc.
Hence a high BMI is a major risk factor for colon cancer. The mechanism by which obesity affects colon cancer prognosis is not entirely clear, but it is thought that hyperinsulinemia and increased insulin-like growth factor (IGF), typical of the obese patient, may be involved in the mechanisms by which obesity confers an increased risk of colon cancer.

Conclusions

In summary, changes in adipose tissue distribution, understood as SAT and VAT, before and after cetuximab therapy were observed in this study, and an increase in visceral adipose tissue (VAT) emerged in treated metastatic colon cancer patients with cetuximab therapy. Unfortunately it is not a clinical indicator of disease but probably the cause is linked to the low number of patients.
In addition, progression-free survival (PFS) was evaluated and the statistical analysis showed that this is better in obese and overweight patients than in normal weight patients.

The role of obesity and especially, visceral fat, in patients with metastatic colon cancer is not fully understood to date. VAT has been clearly associated with an increase in the incidence of colon cancer and its recurrence, as well as negatively affecting the survival of these patients.
Among the deleterious effects of VAT there is an association with chronic inflammation and an increase in circulating pro-inflammatory cytokines, which are involved in a series of diseases related to obesity, such as hypertension, inflammation, atherosclerosis, dyslipidemia, type 2 diabetes, metabolic syndrome, oncological diseases, etc.
Hence a high BMI is a major risk factor for colon cancer. The mechanism by which obesity affects colon cancer prognosis is not entirely clear, but it is thought that hyperinsulinemia and increased insulin-like growth factor (IGF), typical of the obese patient, may be involved in the mechanisms by which obesity confers an increased risk of colon cancer.

Paradoxical effects of obesity on T cell function during tumour progression and blockade of the PD-1 immune checkpoint

Obesity, defined by an increase in BMI (> 30 kg / m2), which reflects the accumulation of visceral fat, is reaching epidemic proportions. Obesity has been associated with numerous comorbidities such as diabetes, heart disease and cancer (Tao, W. & Lagergren, J. 2013) and represents a significant social burden representing > 20% of total annual health care expenditure in the United States (Cawley, J. & Meyerhoefer, C. 2012).

Although obesity is characterized by a "meta inflammatory" state with dysregulated and "inflammatory" immune responses (Hotamisligil, G.S. 2006), little is understood about the impact of obesity on immune responses during cancer progression and immunotherapy. Recent clinical analyzes show that obesity is associated with improved response and survival of cancer patients treated with targeted therapy and checkpoint blockade immunotherapy, although a mechanistic link has not been elucidated (McQuade, J. L. et al. 2018). In Wang's study, Z et al. (Wang Z, et al. 2019) the impact of obesity on T-cell responses was investigated and a significant impact of obesity on the PD- (L) 1 axis, immune aging and dysfunction was demonstrated.

Cancer species and models.

A particular effect of obesity has been shown on tumor progression in mice and on clinical outcomes in cancer patients treated with block of the PD- (L) 1 checkpoint stratified by body mass. PD- (L) 1 signaling is critical both for the initial priming of T cells and for the subsequent depletion of T cells, which occurs with aging or chronic antigen stimulation resulting in impaired proliferative and functional abilities (Wherry, E.J. & Kurachi, M. 2015). Blocking this pathway significantly increases T cell responses in a variety of viral and tumor models (Barbiere, D.L. et al. 2006).

However, despite the success of PD- (L) 1 blockade in multiple malignancies, these therapies fail to generate sustained benefit in most patients. This study highlights the contrasting and paradoxical effects, both positive and negative, of obesity on the immune responses of cancer in the

context of immunotherapy. It is shown that obesity increases the aging of T cells resulting in greater expression and dysfunction of PD-1, which is driven, at least in part, by leptin signaling. An increase in tumor progression has also been observed in the context of obesity, and this is probably due to immunosuppression (Amjadi, F, et al. 2011). However, PD-1-mediated T cell dysfunction in obesity has significantly left tumors markedly more responsive to checkpoint blockade. Importantly, these preclinical results are corroborated by clinical data demonstrating significantly improved outcomes in obese cancer patients treated with PD-1 / PD-L1 inhibitors. Although this study focuses on obesity-induced PD-1-mediated T-cell dysfunction, gender, and other factors such as age, genetics, metabolic dysregulation, gut microbiome, dietary differences, and duration of obesity likely confuse the effects of obesity on the immune system. Furthermore, obesity probably operates through multiple pathways in addition to the PD- (L) 1 axis to induce T cell dysfunction and promote tumor growth, and these phenomena are probably also influenced by diet. Overall, the inflammation associated with obesity causes an increase in the aging of T cells and the induction of normal suppressive pathways to counter this chronic inflammatory state.

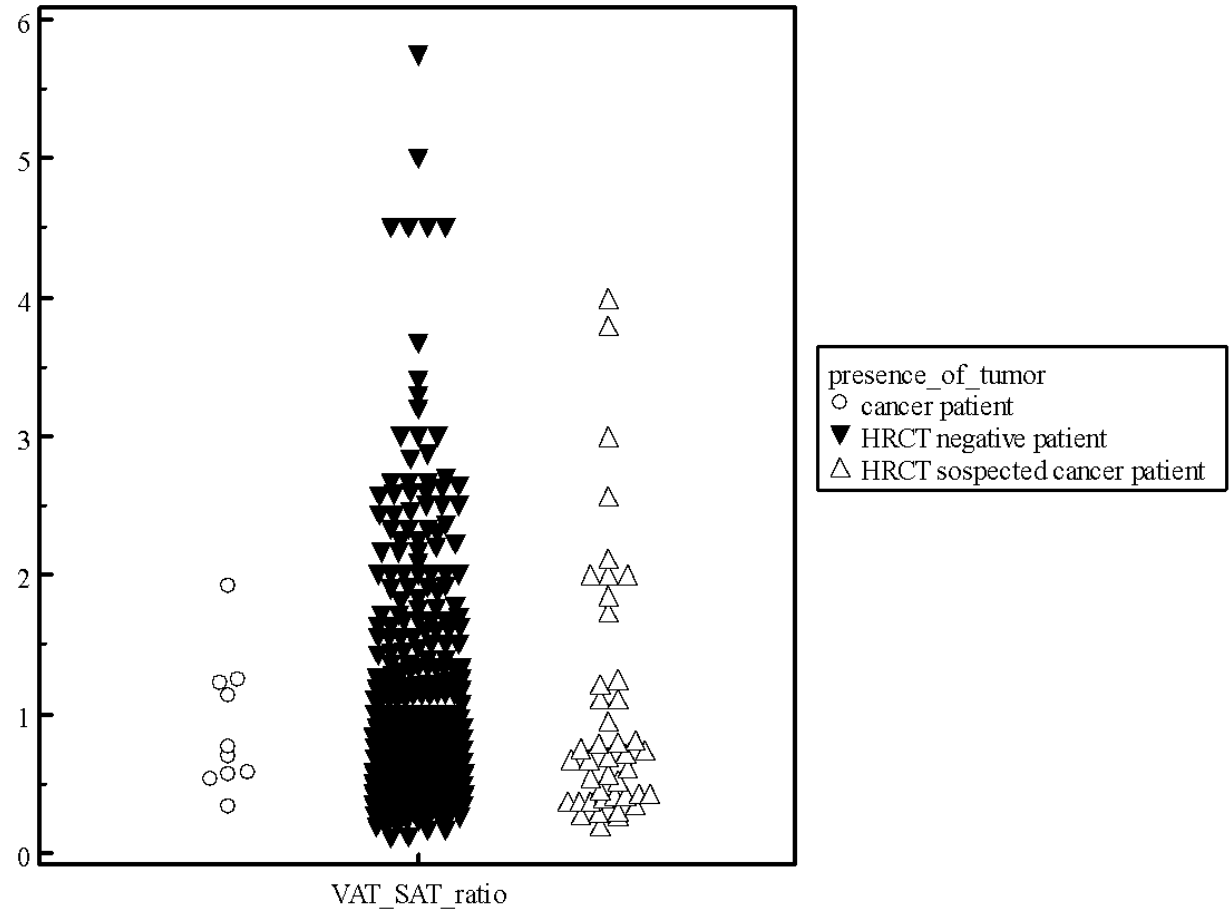
In obesity, PD-1-mediated immune suppression may be a mechanism to protect against possible self-reactive or hyperactive T cell responses induced by chronic inflammation. It is important to emphasize that the link between obesity and leptin and T cell dysfunction and PD-1 in cancer progression appears remarkably robust in both mouse and human studies.

It remains to be determined whether obesity increases PD-1 CD8 T cells in a wide range of human cancers and whether this mechanism also contributes to the increase in cancer incidence in obese patients. Furthermore, it remains to be clinically delineated whether the environment in the obese state results in greater activation and function of T cells once the checkpoint block is applied. It is not clear whether obesity can affect the survival of cancer patients from other non immune factors as well. In this regard, obesity should not necessarily be considered as a positive prognostic factor in cancer, but rather as a potential mediator of immune dysfunction and tumor progression that can be successfully reversed by successful inhibition by checkpoint inhibition. PD- (L) 1 resulting in greater effectiveness.

TEST 5 (a pearl and pitfall thanks to Covid-19)

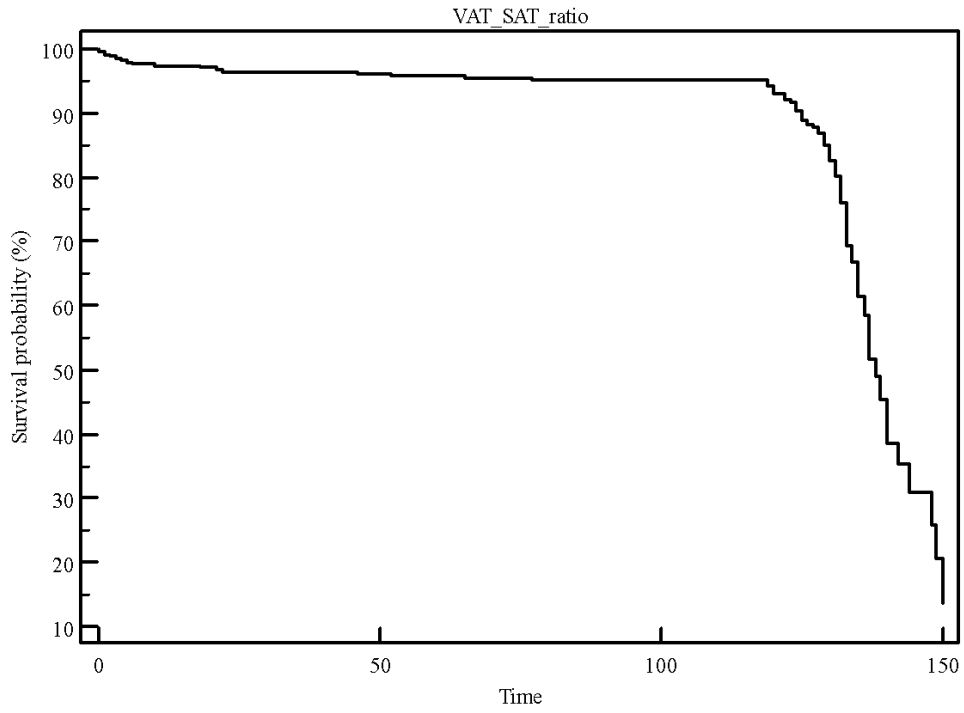
During COVID 2019, the first wave generated a condition to have more CT images available to determinate VAT/SAT ratio as a clinical indicator of mortality for the onset disease, and we retrospectively decided to use it as biomarker. Unfortunately the platform in use during the emergency situation, does not lead to verifying all the clinical information of the patient and the presence or absence of tumor was unknown when data were collected. We correlated only with images (present, absent or doubt disease). This was a single-center cohort study of 441 patients consecutively admitted to the Emergency Department (ED) . Only 9 patients had a positive history of oncological disease. This condition showed the limitation of informatica use in medicine during clinical practice. And the biomarker could not be used for the scope of mortality.

Graph 7



Graph7 is showing the VAT/SAT distribution in HRCT scanner in thoracic images analysis of images on 2D analysis at level between L2-L3. Unfortunately no data available regarding clinical information of therapy were collected for this study.

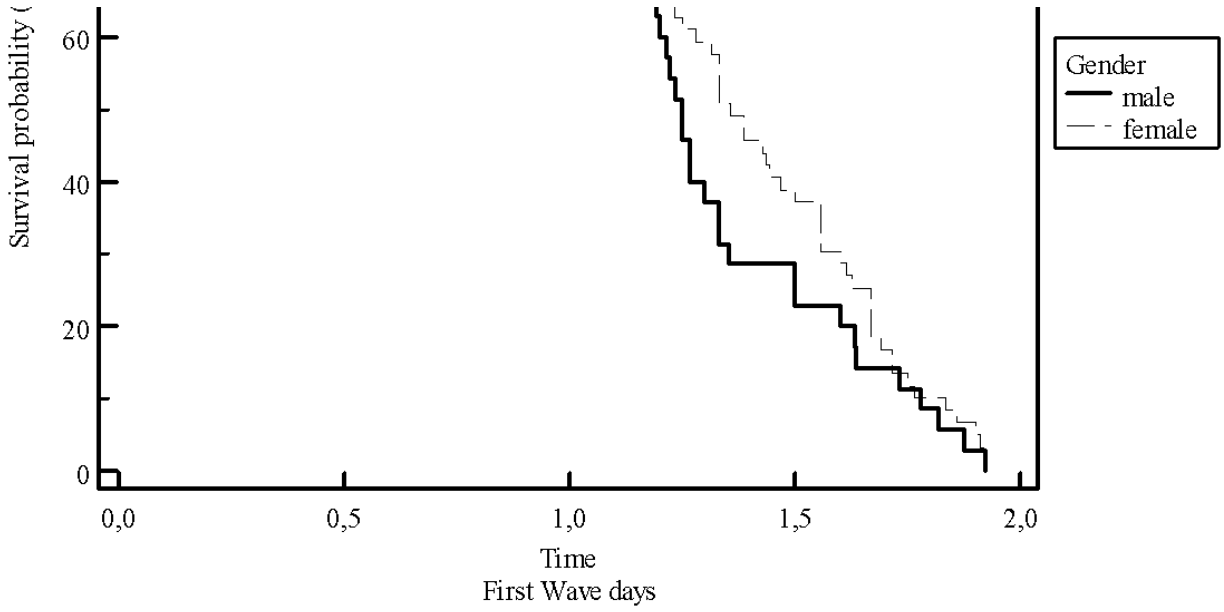
Graph 8



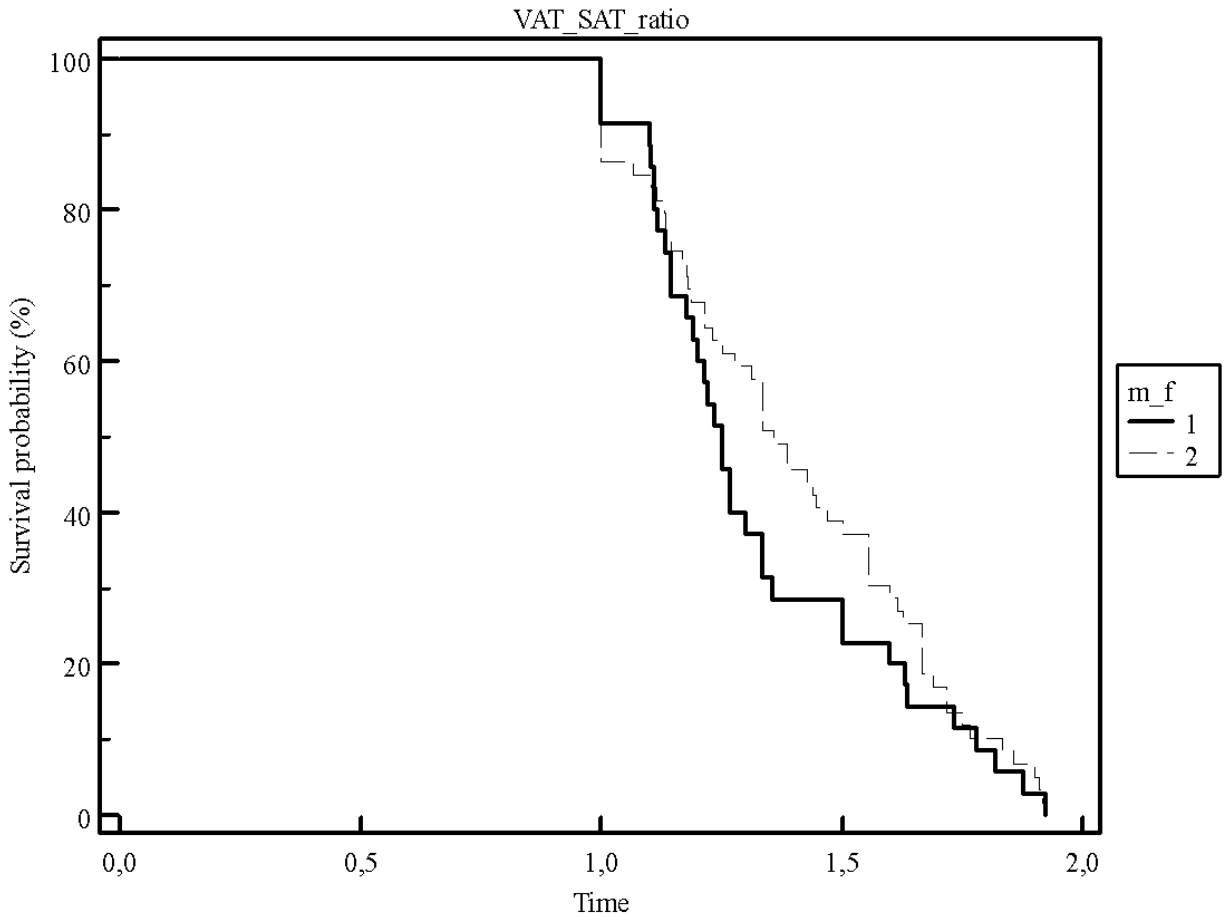
Comparison of survival curves (Log Rank test)

Chi-squared	7,7347
DF	1
Significance	P = 0,0054

Graph 8: In the group of Covid population KM resulting in the end of the study in July during the first wave.



Graph 9 KM showing the higher mortality during the first Covid wave of fat people comparing the normal body shape



Graph 10

Comparison of survival curves (Log Rank test)

Chi-squared	0,9569
DF	1
Significance	P = 0,3280

Final Thesis conclusion: The study result VAT/SAT ratio potentially useful as a "key" indicator for biomarkers in different conditions of disease, unfortunately the data were incomplete to describe variation in oncological patients, the size of the number of patients were not sufficient, more data are needed. Moreover the clinical indicator has importance only if the operator are able to evaluate the clinical situation

at the moment of image acquisition. Variation of VAT/SAT is possible for the same person in a short time. The most important limitation of the study is not possible insert race diversity. Moreover the software complete automatic was not buy in the institution because it was considered too much expensive to test body composition of oncological patients for research scope.

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