UNIVERSITY LA SAPIENZA OF ROME, FACULTY OF MEDICINE

ANGIO-CARDIO-THORACIC PATHOPHYSIOLOGY AND IMAGING

DOCTOR OF PHILOSOPHY, XXXII^EDITION

COORDINATOR PROFESSOR G. VENUTA

New Perspectives in the diagnosis of Cardiac Allograft Vasculopathy: the CT-scan role in the follow-up of heart transplanted patients

---

CANDIDATE

Marzia Cottini, MD

Candidate's ID number: 1748036

Academic Year 2016/2017

INDEX

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVATIONS and ACRONYMS

Abbreviations and Acronyms

16-MDCT = 16-slice multidetector computed tomography
64-MDCT = 64-slice multidetector computed tomography
Ag = antigen
BMI = body mass index
BMS = bare metal stent
BNP = brain natriuretic peptide
bpm = beats per minutes
CAC = coronary artery calcium
CAD = coronary atherosclerotic disease
CAV = cardiac allograft vasculopathy
CCA =coronary artery angiography
CCTA = coronary CT angiography
CD = cluster of differentation
CI = confidence interval
CIN = contrast-material–induced nephropathy
CMV = cytomegalovirus
CNIs = Calcineurin inhibitors
CRP = C-reactive protein
CT = computed tomography
CT-FFR = CT-derived fractional flow reserve
CTDIvol = volume CT dose index
CTP = CT myocardial perfusion
CX = left circumflex
D/GL = death or graft loss
DA: descending anterior coronary
DAP = dose area product
DES = drug eluting stent
DLP = dose-length product
DS = Dual-source
DSE = Dobutamine stress echocardiography
ECG = electrocardiogram
ECM = extracellular matrix
EEM = external elastic membrane
EGF = endothelial grow factor
ELAM =endothelial cell adhesion molecule
eNOS = endothelial NO synthase
FFR = fractional flow reserve
FGF = fibroblast grow factor
GFR = glomerular filtration rate
GP130 = glycoprotein 130
HFmrEF = heart failure mid-range ejection fraction
HFpEF= heart failure preserved ejection fraction
HFrEF = heart failure preserved ejection fraction
HR = hazard ratio
HTx = Heart transplantation
HU = Hounsfield units

ICA = invasive coronary angiography
IFN =interferon
IFN-ɣ = interferon-gamma
IGF = insulin-like grow factor
IL = interleukin
IMR = index of microcirculatory resistance
ISHLT = International Society for Heart and Lung Transplantation
IVUS = intravascular ultrasound
k = organ-weighting factor
LAD = left anterior descending
LM = left main
LVEF = left ventricle ejection fraction
MD = multidetector
MDCT= multidetector computed tomography
MHC = major histocompatibility complex
MIT = maximal intimal thickness
MMF = mycophenolate mofetil
MMP-1=matrix metalloproteinases-1
MPI = myocardial perfusion imaging
mTOR = mammalian target-of-rapamycin
MTS = metal stent
NF-MACE = non-fatal major adverse cardiac events
NO = Nitric Oxide
NPV = negative predictive value
NPV = negative predictive value
OCT = optical coherence tomography
OHT = orthotopic heart transplantation
OPG = Osteoprotegerin
OR = odds ratio
PCI = percutaneous coronary intervention
PDGF=platelet-derived growth factor
PhD = doctor of philosophy
PPV = positive predictive value
PPV= positive predictive value
PTDL = post-transplantation lymphoproliferative disorder
RCA = right coronary artery
Se = sensibility
SMC = small muscle cell
Sp = specificity
SPECT = single-photon emission computed tomography
SSc = systemic sclerosis
TGFβ = transforming growth factor beta
Th1= T helper type 1 cells
TNF-α = tumor necrosis factor-α
Treg = T-regulatory cell
VCAM = vascular cell adhesion molecule
VCAM-1= receptors cell adhesion molecule-1
VH = Virtual histology
vSMC = vascular small muscle cell
vWf = von Willebrand factor

LIST of PAPERS

This is the summary of abstracts/posters presented at national and international congress and the article published regarding my PhD issue.

1. **M Cottini**, FB Fabio Sbaraglia, VB Vitaliano Buffa, PLM Paola Lilla Della Monica, GDS Giada Distefano, AP Amedeo Pergolini, VP Vincenzo Polizzi, FM Francesco Musumeci, *Cardiac allograft vasculopathy: the way for early diagnosis*, European Journal of Heart Failure, Volume 20, Issue S1, 2018.

2. **M Cottini**, FB Fabio Sbaraglia, VB Vitaliano Buffa, PLM Paola Lilla Della Monica, GDS Giada Distefano, AP Amedeo Pergolini, VP Vincenzo Polizzi, FM Francesco Musumeci, *Cardiac allograft vasculopathy: the way for early diagnosis, Heart Failure 2018 ESC Wien, 26-29/03/2018, P1953*

3. **M. Cottini**, Vitaliano Buffa, Vincenzo Polizzi, Fabio Sbaraglia, Giada Di Stefano, P. Myriam Lo Presti, Amedeo Pergolini, Federico Ranocchi, Andrea Montalto, Riccardo Gherli, Emilio Ferretti, Paolo Giuseppe Pino, Paola Lilla Della Monica, Francesco Musumeci. *Cardiac allograft vasculopathy: new perspective in diagnostic workout.* ESC Congress 2017, Barcellona (Accepted abstract and poster presentation 82834)

4. **Cottini M,** Feccia M, Montalto A., Ranocchi F., Luzi G., Gherli R., Ferretti E., Fiorani B., Bergonzini M., Giacopino F., D'Alessandro C., Lo Presti M., Sbaraglia F, Di Stefano G, Pergolini A, Polizzi V, Pino G, Buffa V, Lilla della Monica P, Musumeci F. *Il Trapianto Cardiaco combinazione di arte chirurgica e mente cardiologica. Esperienza quindicennale di un singolo centro. (Abstract Session C24, 28 October 2016, SITO Congress, Rome)*

5. **Cottini M,** Dominici T, Montalto A., Ranocchi F., Luzi G., Gherli R., Ferretti E., Fiorani B., Bergonzini M., Giacopino F., D'Alessandro C., Feccia M, Lo Presti M., Sbaraglia F, Di Stefano G, Pergolini A, Polizzi V, Pino G, Buffa V, Lilla della Monica P, Musumeci F. *Vasculopatia del Cuore Trapiantato: qual è la migliore metodica diagnostico-strumentale per la diagnosi precoce?* (Abstract Section C 04, 27 October 2016, SITO Congress, Rome).

6. Dominici T, MD, **Cottini M**, MD, Sbaraglia F, MD, Della Monica L, MD, Di Stefano G, MD, Pergolini A, MD, Polizzi V, MD, M. Feccia, MD, Fierro S, MD, Buffa V, MD , Musumeci F, MD. *Cardiac Allograft Vasculopathy assessed by 64 slice Dual-Source Coronary Computed Tomographic Angiography: Retrospective Analysis of a Monocentric Experience. (ISHLT Meeting Washington 2016, Accepted, poster presentation 0525).*

7. **Cottini M**, Dominici T, Sbaraglia F, Pergolini A, Di Stefano G, Polizzi V, Feccia M, Buffa V, Lilla Della Monica P, Musumeci F. *Future perspectives in Cardiac Allograft Vasculopathy*. Asian Journal of Science and Technology, Vol.07, Issue, 04, pp.2703-2707, April 2016.

INTRODUCTION

Coronary allograft vasculopathy (CAV) limits long-term survival after heart transplantation, it is documented widely in scientific literature with original articles and reviews.

The screening for CAV is generally performed on an annual or biannual basis. It is usually detected by conventional coronary angiography (CCA) but in the last ten years, Coronary Computed Tomography Angiography (CCTA) has spreading more in more in the study of early detection of CAV due to evolution of technologies.

Technological advances such as 64-slice dual-source CCTA or 128-slice dual-source CCTA might justify re-evaluation of the current recommendation in the detection of CAV.

Inspired by the high quality intravascular CAV detection (IVUS and OCT), I considered the CCTA as new diagnostical procedure with low-technical risk and high technologies and I developed my PhD issue in order to have the following endpoints: improving heart transplant recipient survival and decreasing/controlling the CAV incidence by rapid and early treatment.

I conjectured:

i) Which would be the new perspectives in CAV diagnostic imaging?
ii) Considering the CCTA technological evolution, how could be the comparison with CCA?
iii) How is the comparison with other recommended intravascular diagnostic procedures like IVUS?
iv) Could I create a prognostic score to calculate indirectly the risk of CAV in heart transplanted patients in order to improve its management?

Hence, I had been started my study design to fix my endpoints step by step.

CHAPTER 1: The Heart Transplantation

1.1 Definition of the End Stage Heart Failure

Heart failure (HF) is a clinical syndrome characterized by typical symptoms that may be accompanied by signs caused by a structural and/or functional cardiac abnormality, resulting in a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress [1] [2] (*Table 1*).

Type of HF		HFrEF	HFmrEF	HFpEF
CRITERIA	1	Symptoms ± Signs[a]	Symptoms ± Signs[a]	Symptoms ± Signs[a]
	2	LVEF <40%	LVEF 40–49%	LVEF ≥50%
	3	–	1. Elevated levels of natriuretic peptides[b]; 2. At least one additional criterion: a. relevant structural heart disease (LVH and/or LAE), b. diastolic dysfunction (for details see Section 4.3.2).	1. Elevated levels of natriuretic peptides[b]; 2. At least one additional criterion: a. relevant structural heart disease (LVH and/or LAE), b. diastolic dysfunction (for details see Section 4.3.2).

BNP = B-type natriuretic peptide; HF = heart failure; HFmrEF = heart failure with mid-range ejection fraction; HFpEF = heart failure with preserved ejection fraction; HFrEF = heart failure with reduced ejection fraction; LAE = left atrial enlargement; LVEF = left ventricular ejection fraction; LVH = left ventricular hypertrophy; NT-proBNP = N-terminal pro-B type natriuretic peptide.
[a]Signs may not be present in the early stages of HF (especially in HFpEF) and in patients treated with diuretics.
[b]BNP>35 pg/ml and/or NT-proBNP>125 pg/mL.

Table 1: Definition of the Heart Failure according to the 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution [2]

Nowadays, 5.7 million people in the US have HF, but the projections are worrisome since it is expected that by 2030 more than 8 million people will have this condition, accounting for a 46 % increase in prevalence (see *Figure 1*) [3].

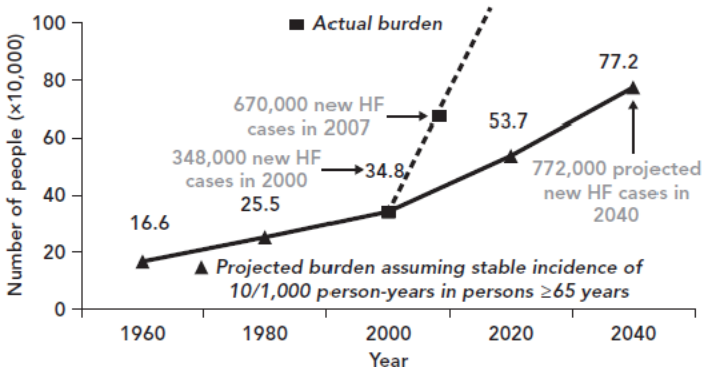


Figure 1: Incidence of HF in the US and the future perspectives

In Europe, the Epidemiologia da Insuficiencia Cardiaca e Aprendizagem (Epidemiology of Heart Failure and Learning – EPICA) study performed in the late 1990s in Portugal reported HF prevalence

of 1.36 % in the 25–49-year-old group, 2.93 % in the 50–59-yearold group, 7.63 % in the 60–69-year-old group, 12.67 % in the 70–79-year-old group, and 16.14 % in patients >80 years [4]. Another analysis in Spain showed HF prevalence steadily increasing from 895 per 100,000 population per year in 2000 to 2,126 cases in 2007, with higher rates in men than women. The prevalence of HFpEF was higher than that of HFrEF; in the former rates were higher in women, while in the latter they were higher in men. The overall HF prevalence significantly increased with ageing, particularly among patients >64 years and with HFpEF [5]. In Germany in 2006 the prevalence of HF was 1.6 % in women and 1.8 % in men, with numbers increasing considerably with advancing age [6]In Sweden in 2010 the crude prevalence of HF was 1.8 % and was similar in men and women, but after adjustment for demographic composition the estimated rate was 2.2 %, with a weak decrease in temporal trend in women but not men between 2006 and 2010 [7]. A recent survey reported HF prevalence of 1.44 % in Italy, with rates increasing with the ageing of the population. HF is also an important health problem in Asia, and its prevalence seems to be even higher compared to Western countries, ranging between 1.3 % and 6.7 % Currently in China there are 4.2 million people with HF, with an estimated prevalence of 1.3 % (see *Figure 2*). [8].

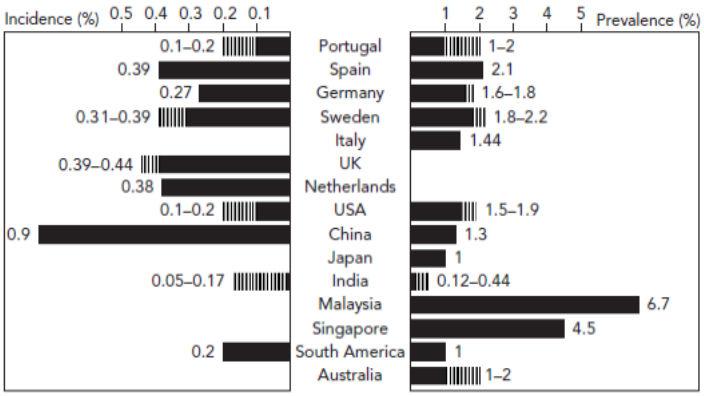


Figure 2: Prevalence and incidence of HF worldwide [9]

HF outcomes have been extensively investigated in the US. The Organized Program to Initiate Lifesaving Treatment in Hospitalized Patients With Heart Failure (OPTIMIZE-HF) study enrolling 20,118 patients with HFrEF and 21,149 with HFpEF (EF ≥40 %) reported no differences between HFrEF and HFpEF in 60–90-day mortality (9.8 % versus 9.5 %) and re-hospitalisation (29.9 % versus 29.2 %), but higher in-hospital mortality in those with HFrEF (3.9 %) versus HFpEF (2.9 %). When the comparison between HFpEF (EF >50 %) and HFmrEF (EF 40–50 %) was performed, no differences in outcomes were observed.[36] Similarly, the Get With The Guidelines (GWTG) registry that enrolled 15,716 patients with HFrEF, 5,626 with HFmrEF and 18,897 with HFpEF observed 37.5 %, 35.1 % and 35.6 % mortality at 1 yearm respectively, with no differences in risk after several adjustments. The 1-year HF hospital readmission rates were 30.9 %, 28.4 % and 24.3 % in HFrEF, HFmrEF and HFpEF, respectively, but there was a higher risk in HFrEF and HFmrEF compared with

HFpEF [10]. The Management Predischarge Process for Assessment of Carvedilol Therapy for Heart Failure (IMPACT-HF) study reported that >50 % of patients were discharged with unresolved symptoms, and within 60 days half had worsening symptoms, a quarter were re-hospitalised and >10 % died [11]. The Canadian Enhanced Feedback for Effective Cardiac Treatment (EFFECT) study enrolling 1,570 patients with HFrEF and 880 with HFpEF reported no differences in mortality at 30 days (7.1 % and 5.3 %, respectively) and 1 year (25.5 % and 22.2 %, respectively). Similarly, for HFrEF and HFpEF there were no differences between HF readmissions at 30 days (4.9 % and 4.5 %, respectively) and at 1-year (16.1 % and 13.5 %, respectively) [12].In Europe, the EuroHeart Failure Survey compared prognosis in 3,148 patients with HFpEF and 3,658 with HFrEF, reporting higher 90-day mortality in those with HFrEF (12 %) compared with HFpEF (10 %), but similar readmission rates (21 % versus 22 %, respectively). In the EuroHeart Failure Survey II, which enrolled 3,580 patients hospitalised for HF, overall in-hospital mortality was 6.4 %.

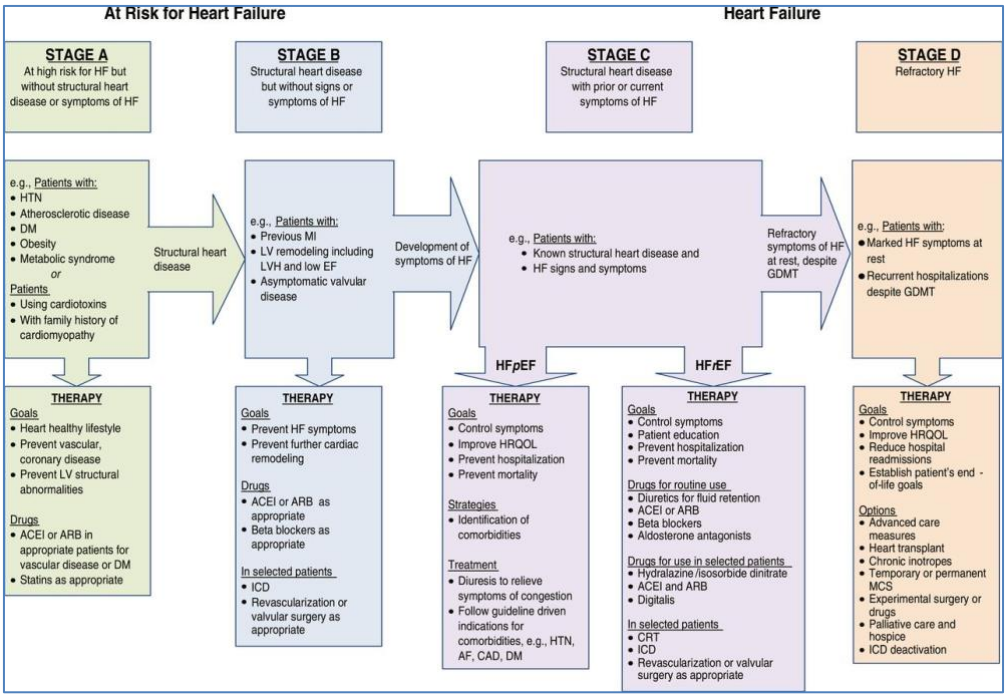


Figure 3: Stages of HF [13]

Recently, in the European Society of Cardiology Heart Failure Long-Term (ESC-HF-LT) registry that enrolled 12,440 patients with acute and chronic HF from 21 European and/or Mediterranean countries, the 1-year mortality rate was estimated to be 23.6 % for acute HF and 6.4 for chronic HF; whereas the rates for the combined endpoint of mortality or HF hospitalisation within 1 year were 36 % for acute HF and 14.5 % for chronic HF. Mortality rates ranged across the different regions from 21.6 % to 36.5 % for acute HF and from 6.9 % to 15.6 % for chronic HF [14]. The HF could

be classified in four stages (see *Figure 3)* [13], with worsening of the hemodynamic and clinical status of the patients from the Stage A to the Stage D.

The end-stage, Stage D, could be defined as the presence of progressive and/or persistent severe signs and symptoms of heart failure despite optimized medical, surgical, and device.

1.2 The Heart Transplantation

1.2.1 Definition

The heart transplantation (Htx) is the gold standard for the unsuccessful medical and surgical therapy in end-stage heart disease (Level of Evidence I C). In the last three years, the number of heart transplantation increased more and more (*see Figure 4*) [15] [16] [17] [2].

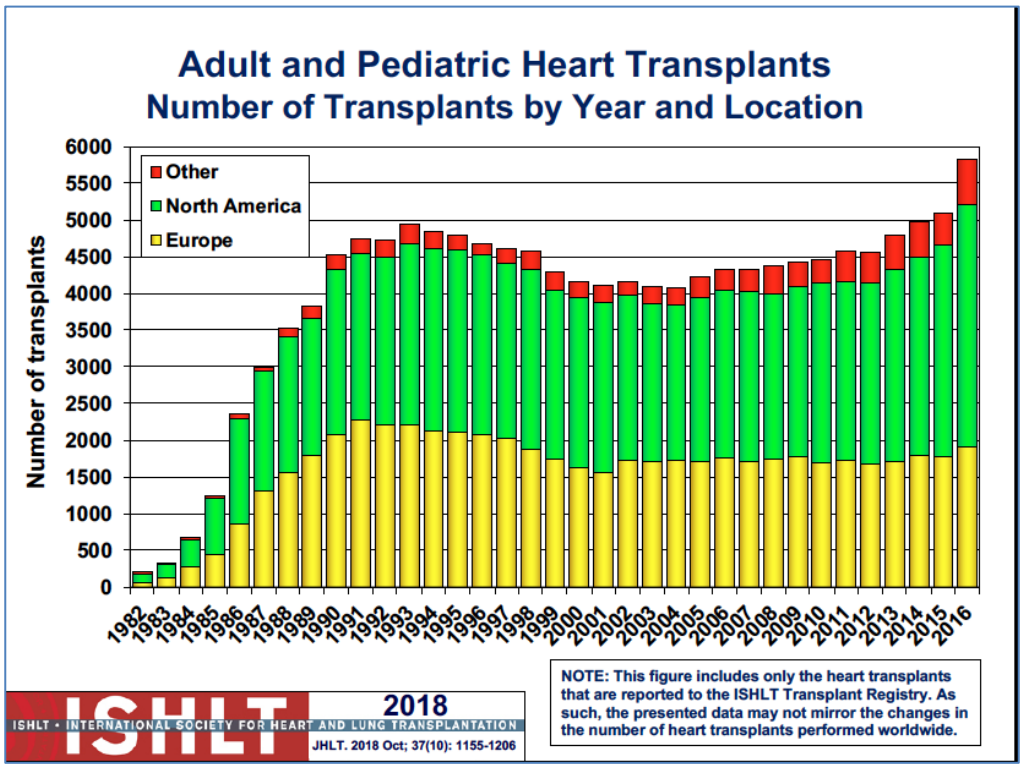


Figure 4: International Society Heart and Lung Transplantation Report of the 2018. The number of HTx procedure since the last 2018 in Europe, North America and other countries

1.2.2 Indications and contraindications of Htx

The main indications and contraindications of the heart transplantation are in the *Table 2*. The indications include [2, 15]:

- ✓ end stage of heart failure with severe symptoms with poor prognosis;
- ✓ end stage of heart failure with no evidence of pulmonary hypertension;
- ✓ refractory cardiogenic shock requiring continuous intravenous inotropic therapy;
- ✓ Peak VO2 (VO2 max) less than 10 ml/Kg per min;
- ✓ NYHA III and IV heart failure symptoms;
- ✓ recurrent life-threatening left ventricular arrhythmias despite an implantable cardiac defibrillator, antiarrhythmic therapy, or catheter-based ablation;
- ✓ refractory angina without potential medical or surgical therapeutic choice.

The Contraindications include [17, 2, 15]:

- ✓ *relative contraindications:*
    - patients with HIV,
    - hepatitis,
    - Chagas disease,
    - tuberculosis,
    - active infection, excluded LVAD-related infection,
    - severe peripherical vascular disease,
    - severe osteoporosis,
    - BMI> 35 Kg/m2,
    - advanced age (more than 65 years old),
    - phycological instability
    - active or recent substance abuse
- ✓ *absolute contraindications:*
    - severe cerebrovascular arterial disease;
    - pharmacologically irreversible pulmonary hypertension,
    - history of solid organ or hematologic malignancy within the last 5 years due to probability of recurrence
    - irreversible renal dysfunction,
    - advanced irreversible liver dysfunction,
    - advanced irreversible pulmonary parenchymal disease or FEV1 < 1 L/min
    - systemic disease with multi-organ involvement;
    - serious comorbidities

Patients to consider	End-stage HF with severe symptoms, a poor prognosis, and no remaining alternative treatment options. Motivated, well informed, and emotionally stable. Capable of complying with the intensive treatment required postoperatively.
Contra-indications	Active infection. Severe peripheral arterial or cerebrovascular disease. Pharmacologically irreversible pulmonary hypertension (LVAD should be considered with a subsequent re-evaluation to establish candidacy). Cancer (a collaboration with oncology specialists should occur to stratify each patient as to their risk of tumour recurrence). Irreversible renal dysfunction (e.g. creatinine clearance <30 mL/min). Systemic disease with multi-organ involvement. Other serious co-morbidity with poor prognosis. Pre-transplant BMI >35 kg/m^2 (weight loss is recommended to achieve a BMI <35 kg/m^2). Current alcohol or drug abuse. Any patient for whom social supports are deemed insufficient to achieve compliant care in the outpatient setting.

Table 2: HTx indications and contraindications according to ESC 2016 HF Guidelines [2].

1.2.3 Surgical Techniques

The Heart transplantation could be performed by different techniques:

- *Orthotopic technique*:

    - *Shumway technique (BIATRIAL technique):* During implantation, perfusate temperature is generally28°C, with intermittent topical cooling using 4°C saline ice slush. No additional cardioplegic solution is infused. The left atrial anastomosis is constructed first using continuous 3-0 polypropylene suture When constructing it, he first few stitches are placed "at a distance" before lowering the donor heart into the pericardial space. The remainder of the entire left atrial anastomosis is constructed in an everting fashion to provide endothelium-to-endothelium apposition, thereby reducing the chance of thrombus formation along the suture line. Construction of the far-leftward portion of the anastomosis along the left pulmonary veins is often facilitated by retracting the donor ascending aorta inferiorly with a traction suture. The right atrial anastomosis is also constructed with continuous 3-0 polypropylene suture. In the area over the interatrial septum, the suture lines are partially overlapping. Each chamber is filled with cold saline before securing the suture lines. The aortic anastomosis is constructed with continuous 4-0 polypropylene suture after the donor and recipient aortas are cut to appropriate length. A cardioplegia catheter to be used as a "needle vent" for aspirating air is placed in the donor ascending aorta. Air is evacuated from the heart through the aortic suture line, and the suture line secured. The aortic clamp is removed with strong suction on the needle vent. When a gentle sinus rhythm is established, preparations are made for the pulmonary artery anastomosis. (Some surgeons prefer to complete this anastomosis before removing the aortic clamp.) The pulmonary artery segments are cut to an appropriate length and the anastomosis constructed, usually with 4-0 or 5-0 polypropylene suture. The remainder of the operation is conducted as usual during rewarming, and CPB is gradually discontinued after thoroughly de-airing the heart through the aortic needle vent while examining it for residual air with TEE.

    - *Shumway procedure (BICAVAL technique):* Orthotopic cardiac transplantation, bicaval technique. Right atrium is divided to create superior and inferior vena caval cuffs. Great vessels are divided as in biatrial method. Commencement of left atrial anastomosis. Completion of bicaval transplant technique, showing inferior vena caval, superior vena caval, aortic, and pulmonary trunk anastomoses

- *Heterotopic technique:* Heterotopic transplantation. Donor superior vena cava is anastomosed end to side to recipient superior vena cava. Anastomosis may be facilitated by transient removal of superior vena caval cannula. Aortic anastomosis completed. Pulmonary artery connection requires interposition of a polyester graft.

1.2.4 Survival

According to The International Society for Heart and Lung Transplantation estimates that more than 5,000 heart transplants are performed each year worldwide. The long-time survival of heart transplanted patients is 87.8%, 78.5% and 71.7% at 1, 3 and 5 years after surgery respectively (*see Figure 5-6)* [18, 19, 20, 21].

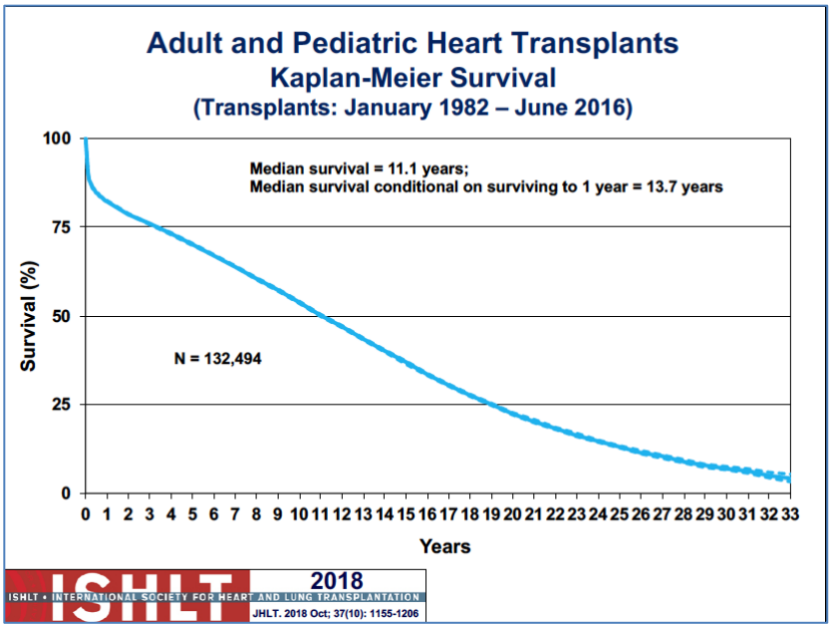


Figure 5: Adult and pediatric heart transplant Kaplan-Meyer cumulative survival curve

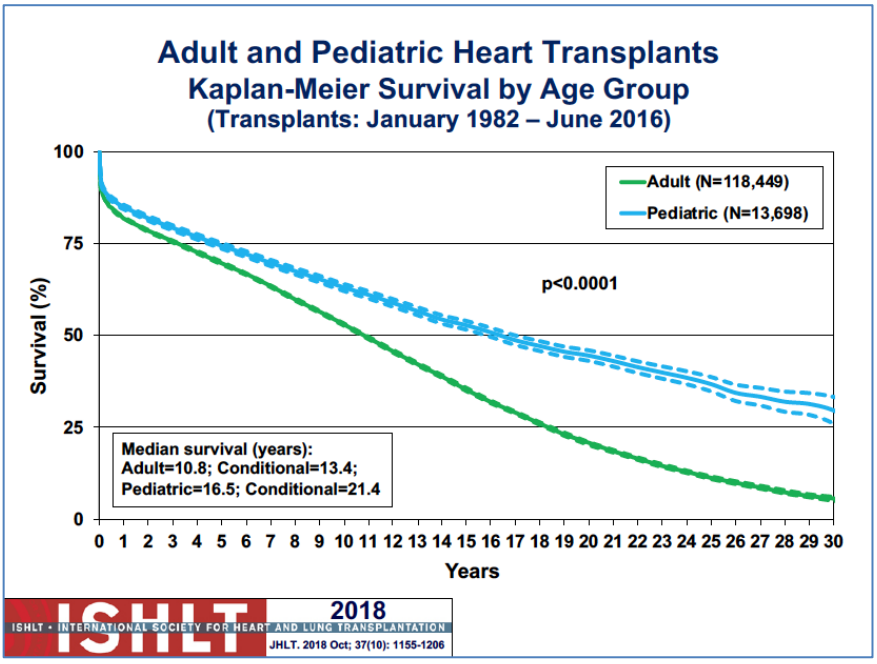


Figure 6: Adult and pediatric heart transplant Kaplan-Meyer group survival curves

1.2.5 Complications

There are a lot of studies documented the management of heart transplant recipient in the postoperative period, short- and long-term. According to ISHLT registry and the most important articles in scientific [22] [23] [24] [9] [25, 26] , the main *short-term complications* are:

- early graft failure and primary graft dysfunction: they are the most common cause of short-term mortality after heart transplantation. Early graft failure (EGF), defined as a composite of death and/or re-transplantation associated with graft failure during the first 30 days after transplant, is the most severe form of primary graft dysfunction (PGD) and constitutes the most feared complication. The incidence of EGF reported for transplants performed between 2005 and 2013 was 3.8%, with a 96.3% mortality rate and 3.6% requiring re-transplantation.
- rejection: *Hyperacute rejection* is mediated by preexisting antibodies to allogeneic antigens and occurs immediately after transplantation with rapid graft failure. It is uncommon because of the current blood- and antigen-typing techniques. *Acute rejection* could be cellular or antibody-mediated rejection, with an incidence of deaths of 8% after heart transplantation. The figure 7 showed the reduction of rejection and treatment in the last years according to ISHLT registry, it documented the decreasing of its incidence due to early diagnosis and treatment.

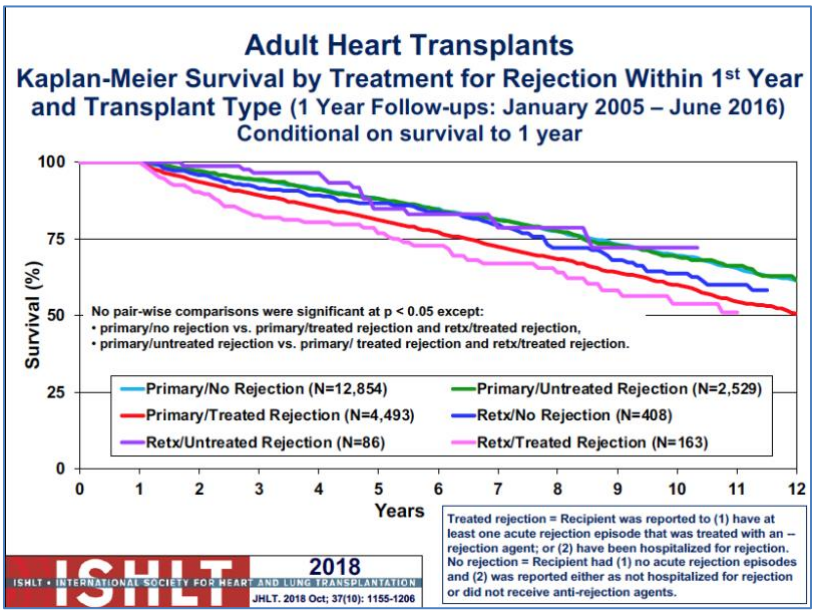


Figure 7: Kaplan-Meyer Survival Curves in Adult Heart transplantation by treatment for rejection within 1st year.

- neurological complications: the rate of cerebrovascular accident (CVA) after heart transplant is reported up to 13%, and is associated with increased mortality post-transplant. CVAs can

be defined as either ischemic or hemorrhagic, with ischemic CVAs being twice as common as hemorrhagic CVAs after heart trans- plantation

- respiratory complications: the most of patients with advanced heart failure have considerable changes in pulmonary function, including abnormal pulmonary diffusion, evident by decreased diffusing capacity of the lungs for carbon mon- oxide. Gas exchange impairment persists in 67% of patients after transplantation, independent of smoking status, prior drug use, chest radiographic changes, hemodynamic findings, or duration of heart failure

The long-term complications are reported by ISHLT 2018 report in the Figure 8.

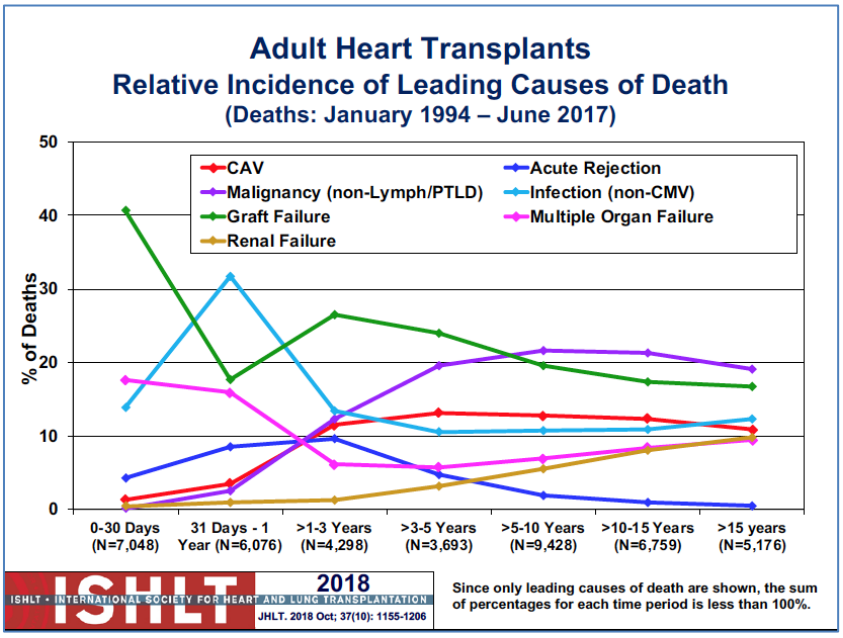


Figure 8: Causes of death after heart transplantation during the post-transplantation

- *Infections* are common. The kind of infection in cardiac transplant recipient is vary, depending on time from transplantation [17]
- *Chronic kidney disease*, the calcineurin inhibitors can induce nephrotoxicity by a decrease in glomerular filtration rate (GFR), afferent arteriolopathy and striped tubulointerstitial fibrosis [27].
- *Endocrine disease:* While diabetes mellitus remains a common comorbidity in patients with advanced heart failure undergoing heart transplantation, hyperglycemia due to chronic steroid use may result in a new diagnosis of diabetes post-transplantation in up to 23 e 39% of patients in the first 2 years

- *Malignancy* is one of the major causes of long-term mortality in heart transplant recipient; the cutaneous ones are the most common but the post-transplantation lymphoproliferative disorder (PTDL) is a frequent fatal complication with high association to Ebstein-Barr virus.
- *Cardiac allograft vasculopathy (CAV)* is the largest long-term complication in the heart transplant recipient, which has and had focused the research and the new digital technologies to improve the early detection in order to increase the patient and graft survival.

The heart transplant recipient causes of death are several, and according to the last ISHLT 2018 report the most relevant are (*see* Figure 9):

Adult Heart Transplants
Cause of Death (Deaths: January 1994 – June 2017)

Cause of Death	0-30 Days (N=7,048)	31 Days - 1 Year (N=6,076)	>1-3 Years (N=4,298)	>3-5 Years (N=3,693)	>5-10 Years (N=9,428)	>10-15 Years (N=6,759)	>15 Years (N=5,176)
Cardiac Allograft Vasculopathy	90 (1.3%)	212 (3.5%)	494 (11.5%)	483 (13.1%)	1,201 (12.7%)	834 (12.3%)	560 (10.8%)
Acute Rejection	294 (4.2%)	516 (8.5%)	413 (9.6%)	172 (4.7%)	177 (1.9%)	62 (0.9%)	28 (0.5%)
Lymphoma	2 (0.0%)	64 (1.1%)	104 (2.4%)	115 (3.1%)	312 (3.3%)	183 (2.7%)	109 (2.1%)
Malignancy, Other	4 (0.1%)	151 (2.5%)	529 (12.3%)	720 (19.5%)	2,036 (21.6%)	1,438 (21.3%)	985 (19.0%)
CMV	3 (0.0%)	58 (1.0%)	21 (0.5%)	6 (0.2%)	8 (0.1%)	4 (0.1%)	2 (0.0%)
Infection, Non-CMV	981 (13.9%)	1,928 (31.7%)	574 (13.4%)	389 (10.5%)	1,006 (10.7%)	736 (10.9%)	638 (12.3%)
Graft Failure	2,858 (40.6%)	1,074 (17.7%)	1,137 (26.5%)	888 (24.0%)	1,835 (19.5%)	1,176 (17.4%)	862 (16.7%)
Technical	500 (7.1%)	93 (1.5%)	31 (0.7%)	28 (0.8%)	94 (1.0%)	81 (1.2%)	68 (1.3%)
Other	312 (4.4%)	401 (6.6%)	338 (7.9%)	281 (7.6%)	719 (7.6%)	449 (6.6%)	381 (7.4%)
Multiple Organ Failure	1,243 (17.6%)	964 (15.9%)	261 (6.1%)	209 (5.7%)	650 (6.9%)	571 (8.4%)	486 (9.4%)
Renal Failure	30 (0.4%)	53 (0.9%)	57 (1.3%)	114 (3.1%)	516 (5.5%)	538 (8.0%)	509 (9.8%)
Pulmonary	189 (2.7%)	230 (3.8%)	175 (4.1%)	164 (4.4%)	429 (4.6%)	318 (4.7%)	252 (4.9%)
Cerebrovascular	542 (7.7%)	332 (5.5%)	164 (3.8%)	124 (3.4%)	445 (4.7%)	369 (5.5%)	296 (5.7%)
Total Deaths (N)	8,121	6,979	5,276	4,647	12,489	9,763	7,735

ISHLT • INTERNATIONAL SOCIETY FOR HEART AND LUNG TRANSPLANTATION 2018
JHLT. 2018 Oct; 37(10): 1155-1206

Percentages represent % of deaths in the respective time period. Total number of deaths includes deaths with unknown causes.

Figure 9: The causes of death after heart transplantation according to ISHLT registry and report 2018.

- at 1 year: Infection non-CMV, graft failure, multiple organ failure
- at 1-3 years: CAV, malignancy, graft failure, infection non-CMV
- at 3-5 years: CAV, malignancy, graft failure
- at 5-10 years: CAV, malignancy, graft failure
- at 10-15 years: CAV, malignancy, graft failure
- more than 15 years: CAV, malignancy, graft failure

Considering the long-term outcome (see Figure 9-10), the most frequent complications are coronary allograft vasculopathy (CAV) and neoplasm. CAV occurs in approximately 12,7% of patients by 5 years and 12,3% by 10 years, it is one of the major causes of graft loss and death.

CHAPTER 2: Cardiac Allograft Vasculopathy

2.1 Definition

Coronary heart disease of the transplanted heart (CAV) is characterized by characterized by intimal proliferation, develops early after trans- plant, is progressive, and accounts for major morbidity and mortality late in the transplant natural history [16, 28].

Also CAV was defined by ISHLT as:

a). A "Primary Vessel" denotes the proximal and Middle 33% of the left anterior descending artery, the left circumflex, the ramus and the dominant or co-dominant right coronary artery with the posterior descending and posterolateral branches.
b). A "Secondary Branch Vessel" includes the distal 33% of the primary vessels or any segment within a large septal perforator, diagonals and obtuse marginal branches or any portion of a non-dominant right coronary artery.
c). Restrictive cardiac allograft physiology is defined as symptomatic heart failure with echocardiographic E to A velocity ratio > 2 (>1.5 in children), shortened isovolumetric relaxation time (<60 msec), shortened deceleration time (<150 msec), or restrictive hemodynamic values (Right Atrial Pressure >12mmHg, Pulmonary Capillary Wedge Pressure >25 mmHg, Cardiac Index <2 l/min/m2)

2.2 Classification

Initially, it was described by Gao [28] and coded anatomic abnormalities into type A, B1, B2, and C lesions (*see* Figure 11).

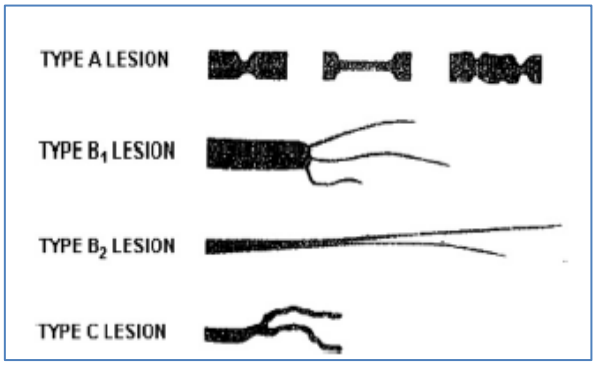


Figure 10: The Classification of CAV according to Gao [28]

- Type A was discrete or tubular stenosis and multiple stenoses in the proximal, middle, or distal segment branches;

- type B1 was a proximal vessel maintaining nor- mal diameter with abrupt onset of distal concentric narrowing and obliteration;
- type B2 was a gradual transition from the normal proximal vessel with tapering, concentric narrowing progressively increasing in severity distally; and type C was a diseased vessel, diffusely irregular that lost small branches with terminations often non-tapered, squared off, and ending abruptly.

Recently, the ISHLT published a new and complete classification of the CAV according to the

RECOMMENDED NOMENCLATURE FOR CARDIAC ALLOGRAFT VASCULOPATHY

ISHLT CAV0 (Not significant): No detectable angiographic lesion

ISHLT CAV1 (Mild): Angiographic left main (LM) <50%, or primary vessel with maximum lesion of <70%, or any branch stenosis <70% (including diffuse narrowing) without allograft dysfunction

ISHLT CAV2 (Moderate): Angiographic LM ≤50%; a single primary vessel ≥70%, or isolated branch stenosis ≥70% in branches of 2 systems, without allograft dysfunction

ISHLT CAV3 (Severe): Angiographic LM ≥50%, or two or more primary vessels ≥70% stenosis, or isolated branch stenosis ≥70% in all 3 systems; or ISHLT CAV1 or CAV2 with allograft dysfunction (defined as LVEF ≤45% usually in the presence of regional wall motion abnormalities) or evidence of significant restrictive physiology (which is common but not specific;)

The Journal of Heart and Lung Transplantation, Volume 29, Issue 7 (July 2010), p 717-727

Figure 11: Nomenclature for Cardiac Allograft Vasculopathy according to ISHLT

2.3 Pathogenesis

CAV is an accelerated fibroproliferative disease affecting the vasculature of the transplanted heart. Pathologically, smooth muscle proliferation, accumulation of inflammatory cells, and lipid deposition cause circumferential intimal thickening. In contrast to the focal, eccentric, proximal epicardial lesions in atherosclerosis, CAV is diffuse and affects epicardial and intramural vessels (*see Figure 12)* [29].

Intravascular imaging has shown disease occurs within the first year of trans- plant, and has a biphasic response, involving initial intimal thickening with expansion of the external elastic membrane and relative preservation of luminal area, followed by constrictive remodeling and luminal narrowing. Plaque composition changes from early fibrous and fibrofatty tissue to late atheromatous necrotic core and calcification [29].

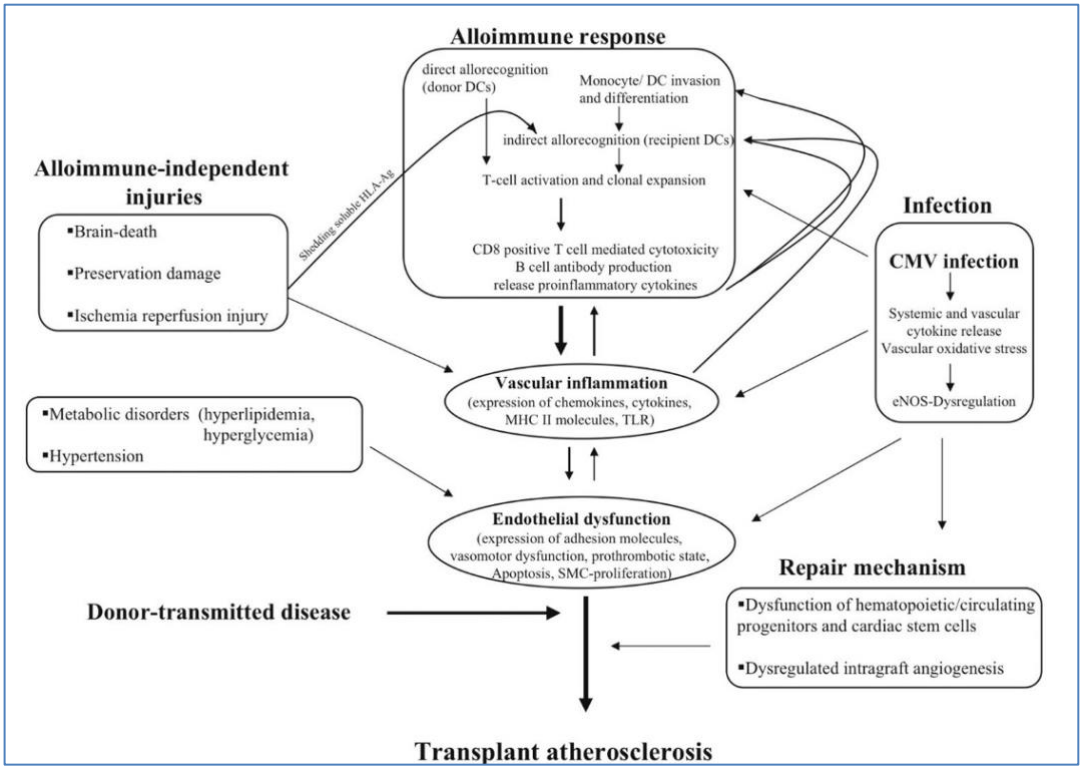


Figure 12: Collaboration and interaction of alloimmune-dependent and -independent factors influencing the pathogenesis of transplant vasculopathy. Ag indicates antigen; CD, cluster of differentiation; eNOS, endothelial NO synthase; and SMC, smooth muscle cell [29].

2.4 Histopathological presentation

Coronary vasculopathy of the transplanted heart (CAV) has typical anatomic-pathological features that significantly differentiate it from coronary atherosclerosis affecting the general population, but that unite it to the lesions detectable in chronic rejection that affects the other transplanted organs.

As for the type of vessels involved, CAV occurs at the level of the whole coronary tree, affecting both epicardial arteries and intramural vessels; it can also occur at the level of the coronary veins, while the vessels without smooth muscles are spared.

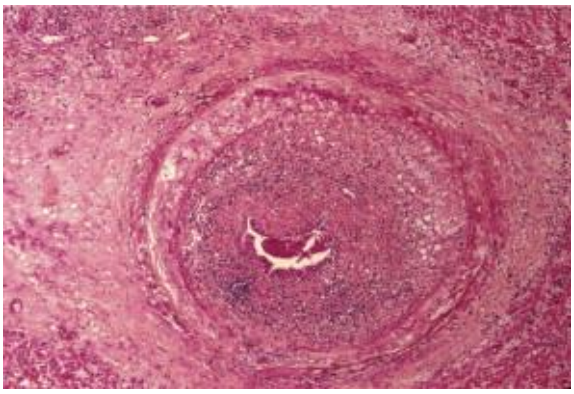


Figure 13: Histological view of the cardiac allograft vasculopathy. It shows the typical concentric lesion versus the eccentric one of atheromatous disease.

The lesions occur uniformly over the entire length of the vessels involved and it has been shown, thanks to a series of autopsy studies, how the severity of the same is comparable between the proximal and distal portions of the epicardial arteries, both as regards the percentage of surface concerned that due to the extent of intimal thickening. (*see Figure 13).*

Atherosclerosis, on the other hand, is characterized by focal and eccentric lesions that almost exclusively affect the proximal portion of the epicardial arteries, saving the intramyocardial circulation and coronary veins (*see Figure 14)*.

The times of appearance of these two processes differ significantly; atherosclerotic lesions at the level of the native heart develop slowly starting from puberty and in most cases they occur clinically only after some decades.

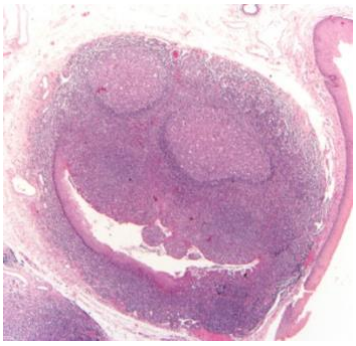


Figure 14: atherosclerotic disease of coronary artery.

In CAV, on the other hand, the first changes in the underwear take place already in the first weeks after the transplant; these initial lesions are characterized by a slight diffuse and concentric thickening of the intima, given by the presence of an inflammatory subendothelial infiltrate of lymphocytes and macrophages, by the proliferation of vascular smooth muscle cells (vascular smooth muscle cells, VSMCs) migrated into the intimate and from the presence of mild fibrosis and increased extracellular matrix proteins.

As the months progress, there is the appearance of intermediate lesions, developed following the accumulation of foam cells (foamy macrophages) and lipids in the intimate area and the accelerated intimal proliferation of modified VSMCs and fibroblast, and of atheromatous plaques, with a core well-formed lipid consisting of cholesterol and lipid residues.

In the long term these intimate fibrous and fibrolipid lesions lead to a picture of concentric and diffuse fibrous intimal thickening and to possible fibrous and fibroadipose plaques (atherosclerotic plaques mixed with a diffuse intimal thickening are late).

The histopathological presentation of these plaques is similar to that found in coronary vasculopathy, but the incidence of complications, such as plaque ulceration and thrombus formation, is very rare, as are the calcification phenomena.

Another difference compared to normal atherosclerosis, which affects all layers of the vascular wall with destruction of the internal elastic lamina, is the fact that CAV is a pathological process that mainly involves the intimate tunic of the vessels; the internal elastic lamina remains, in fact, relatively intact, while the medium tunic and the adventitia, not affected by aggressive intimal proliferation, are progressively replaced by fibrous tissue. The magnitude of this fibrotic process increases as the severity of the pathological process affecting the intima increases.

2.5 Epidemiology and Aetiology

Both the immunologic and non-immunological factors are thought to contribute to the pathogenesis of CAV, still not fully understood; it seems that the former play a fundamental role in the onset of the disease, while the latter favor its progression and spread along the vascular tree *(see Figure 15)* [30].

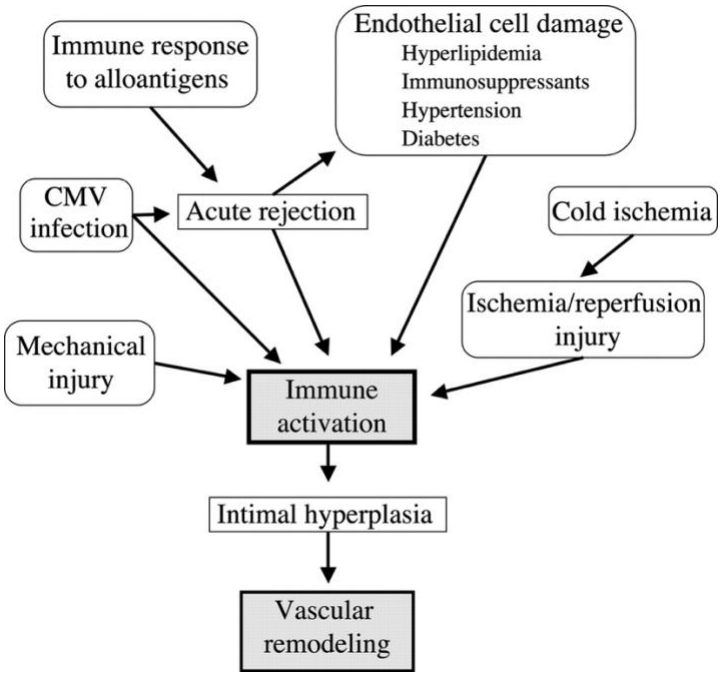


Figure 15: Costimulatory molecules play crucial roles in this T cell activation. Many costimulatory pathways have been described, and some are involved in the pathogenesis of CAV, atherogenesis, and subsequent plaque formation. In this review, we summarize the present knowledge of the role of these pathways in CAV development and the possibility of manipulating these pathways as a means to treat heart allograft vascular disease and atherosclerosis [30]

Regarding the immunological risk factors, the degree of HLA incompatibility between donor and recipient and the number and duration of acute rejection episodes are important. In particular, a study published in 2004 identified a high Rejection Score (RS) for severe rejections (grade ≥3A) as an independent predictive factor for the onset of CAV.

Non-immunological risk factors include the donor's mode of death, ischemia and reperfusion injury, cytomegalovirus infection, age, sex and high donor and recipient weight, as well as common risk factors such as atherosclerosis, dyslipidemia, hyperhomocysteinemia, arterial hypertension, diabetes mellitus and cigarette smoking.

All these risk factors cause or contribute to the maintenance and perpetuation of a coronary endothelial dysfunction, which constitutes the *primum movens* and is fundamental in the pathogenesis of CAV.

This pathological process affecting the graft begins even before explantation, since it has been shown that a sudden brain death causes an increase in the circulating levels of catecholamines, inflammatory cytokines, chemokines and adhesion molecules at the level of the organ vessels to be transplanted; this cascade of events causes an inflammatory response in the heart, resulting in vascular damage.

In the perioperative phase, *ischemia and reperfusion damage* play an important role in the development of endothelial dysfunction.

The extent of the damage caused depends not only on the time of ischemia, on the quality of conservation of the organ during transport, on the hemodynamic state of the donor and on the possible need for inotropic support with catecholamines, but also, paradoxically, from the same reperfusion.

In the initial stages of this process, oxygen free radicals are formed which compromise the endothelium's ability to release nitric oxide, altering the coronary vascular tone.

The same free radicals also activate the leukocytes and macrophages of the host which, in turn, give rise to a vicious circle, through the production of additional free radicals, proinflammatory cytokines and chemokines. Ischemia and reperfusion damage also causes activation of endothelial cells, with an increase in the expression of adhesion molecules, stimulates platelet adhesion, complement activation and proliferation of vascular smooth muscle cells.

All of these processes lead to endothelial dysfunction resulting from ischemia and reperfusion injury.

However, the main determinant in the pathogenesis of CAV appears to be the recipient's immune response to the transplanted organ, and in particular the graft endothelium. These endothelial cells therefore play a key role in the development of this atypical coronary vasculopathy, as they act as both antigen-presenting cells (APCs) and as a target for the immune response that they themselves helped to trigger.

The increased expression of class I alloantigens of the major histocompatibility complex (MHC) expressed by graft endothelial cells is directly recognized by CD8 + T lymphocytes, resulting in cytokine secretion and further activation of endothelial cells. Activated endothelial cells express increased levels of MHC class II antigens that late activate CD4 + T lymphocytes.

This may explain the predominance of CD8 + T lymphocytes in early vascular lesions and the increased proportion of CD4 + T lymphocytes in the advanced stages of the disease. The thus activated T cells release several cytokines, including IL-2, IL-4, IL-5, IL-6, INF-γ, TNF-α and TNF-β, which, others, stimulate cell clone proliferation T alloreactive, stimulate the expression of further

cytokines and adhesion molecules (VCAM-1, ELAM-1). Thanks to the chemotactic action of cytokines and adhesion molecules, it follows the recruitment and accumulation of macrophages and lymphocytes activated at the level of the vascular wall.

These cells secrete various growth factors, such as Platelet derived Growth Factor (PDGF), Insulin-like Growth Factor 1 (IGF-1), Fibroblast Growth Factor (FGF), Epidermal Growth Factor (EGF) and Transforming Growth Factor-β (TGF-β), which cause an intimal migration and an uncontrolled proliferation of modified vascular smooth muscle cells, associated with an increased production of extracellular matrix *(see Figure 16)* [31].

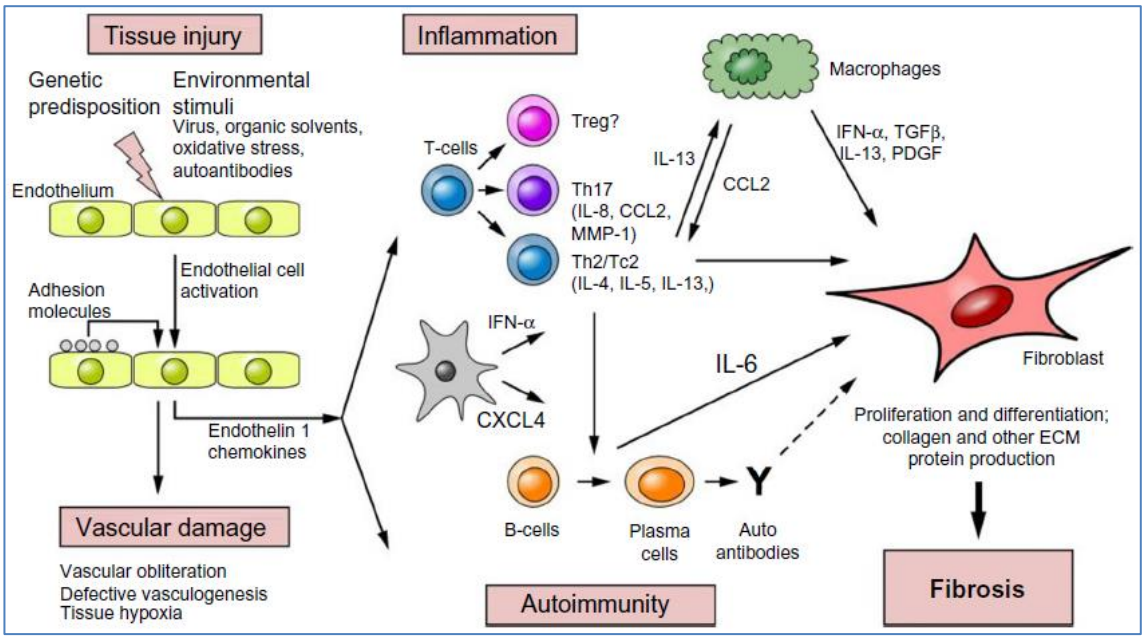


Figure 16: Immunological events involve in the CAV pathogenesis. Environmental and genetic factors contribute to the etiology of SSc. The pathogenesis of SSc involves an interplay between vascular, immunological, and fibrotic processes. Vascular injury and endothelial damage are the earliest events in the pathogenesis of SSc. Activated endothelial cells upregulate the expression of adhesion molecules and secrete chemokines, leading to inflammation and autoimmunity. Macrophages and T-cells are the predominant inflammatory cell types of the inflammatory infiltrates and produce cytokines and growth factors that drive the synthesis of extracellular matrix proteins by fibroblasts, resulting in progressive fibrosis. T-cells have also been implicated in autoantibodies production. [31].

CHAPTER 3:

Detection and imaging in Cardiac Allograft Vasculopathy

3.1 Detection of CAV

In the early era of cardiac transplantation, the diagnosis of CAV was made pathologically.

Angiographic diagnosis emerged rapidly and remained the most important diagnostic tool.

The development of IVUS allowed for detection of early stage CAV not identified by invasive coronary angiography.

In later years, circulating immunehistologic markers as well as gene-based and protein-based biomarkers have been studied to see if they can contribute to grading or detecting CAV.

Routine surveillance is important because HTX patients frequently are asymptomatic, particularly in the early stages of the disease.

Surveillance includes both evaluation of graft function and visualization of the coronary arteries.

Echocardiography is the first-line imaging modality to assess graft function and is part of all serial evaluations during post-transplant follow-up.

With echocardiography, CAV is detected in a late stage when reduced coronary blood flow has resulted in allograft dysfunction.

Dysfunction first manifests as diastolic dysfunction with restrictive physiology, then as systolic dysfunction with reduced ejection fraction.

To detect the presence of CAV and identify potential significant stenosis eligible for intervention, annual or biannual screening with ICA is the current standard of care.

A number of other non-invasive and invasive imaging modalities are used for CAV evaluation (see Figure 17).

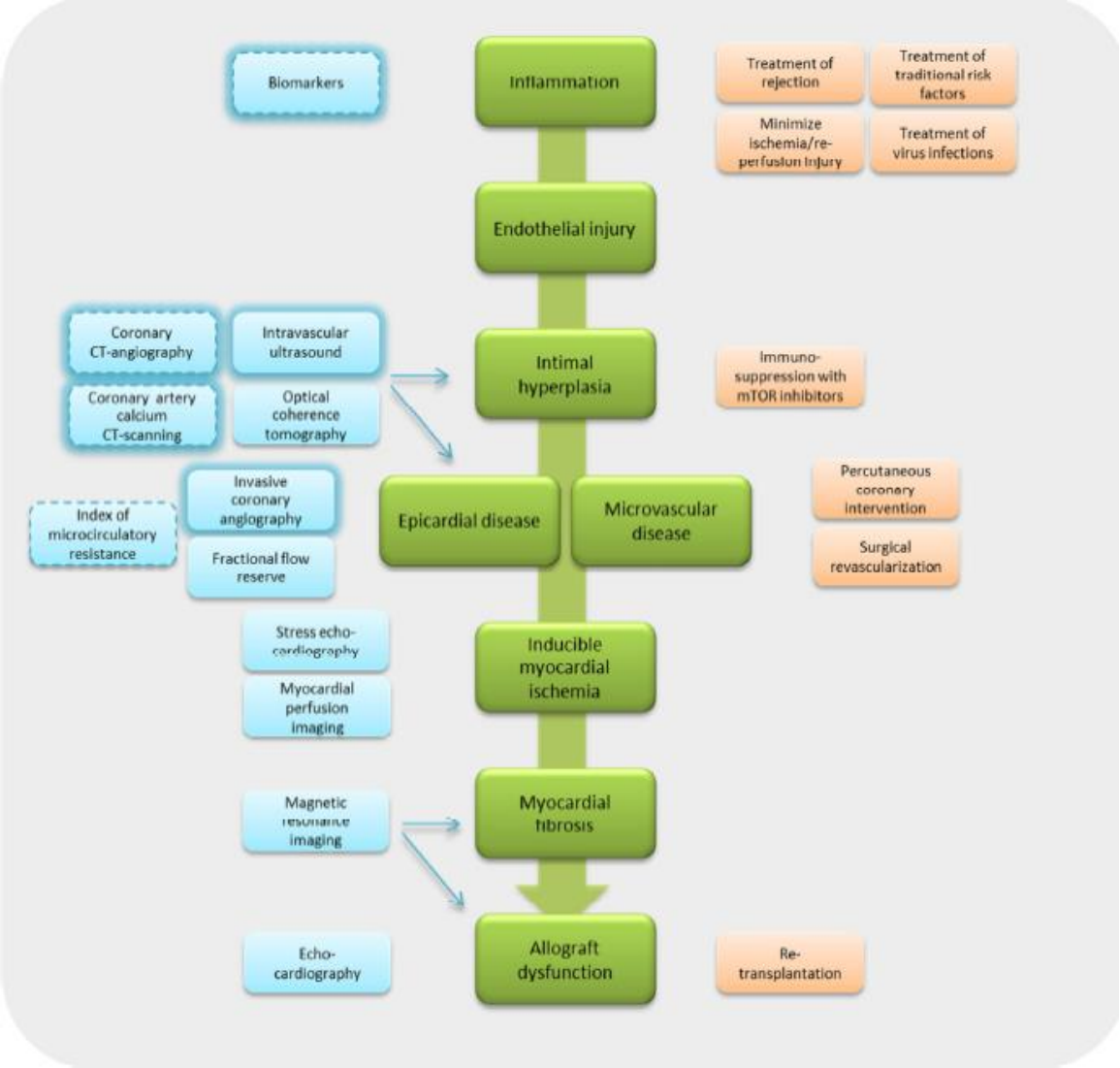


Figure 17: The role of imaging in the detection of CAV in its different stages.

3.2 Non Invasive stress testing

Detection of CAV is challenging with non-invasive techniques, especially in the early stages [32, 33, 34, 35]. Various non-invasive techniques are used for CAV evaluation.

Stress Echocardiography

Dobutamine stress echocardiography (DSE) is commonly used for CAV screening [36, 37] [38]. The surveillance recommendations of ISHLT considered the DSE of Class IIa. DSE gives the information of:

- cardiac structure and function
- regional wall motion
- myocardial deformation
- coronary flow reserve

DSE has an important limitation: it is directly dependent on acustic window.

Myocardial Perfusion Imaging

The myocardial perfusion imaging (MPI) has shown prognostic value and a moderate diagnostic accuracy in the investigation of CAV [39, 40]. Promising results have been demonstrated for MPI with single-photon emission computed tomography (SPECT, Surveillance Recommendation: Class IIa). The SPECT:

- identifies the myocardial perfusion, ventricular function
- its limitation: radiation exposure

Positron emission tomography (PET, Surveillance Recommendation: not included) is the MPI with the most diagnostic accuracy due to its prognostic value and flow quantification (FQ). The FQ is better able to detect the microvascular or diffuse disease. The PET:

- documents myocardial perfusion, myocardial flow quantification and ventricular function
- its strengths: quantify global/regional myocardial blood flow, quantify global/regional myocardial flow reserve
- its limitations: limited availability, radiation exposure

both positron emission tomography and in magnetic resonance imaging in small studies.

Cardiac Magnetic Resonance

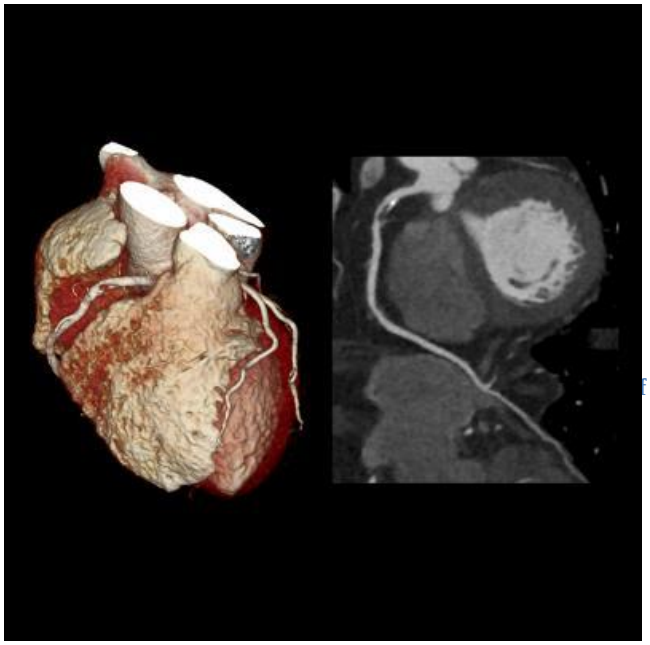
The above-mentioned techniques evaluate myocardial structure, function, and/or perfusion by gadolinium enhancement [41, 42]. Cardiac MRI is:

- safety

- with limitations: high resting rates post-transplant, cardiac device contraindicated, challenging perfusion quantification software, nephrogenic systemic fibrosis in renal failure.

Cardiac Computed Tomography Angiography (CCTA)

CCTA is the only non-invasive technique assessing the coronary arteries, their lumen and wall. In the most recent International Society for Heart and Lung Transplantation (ISHLT) guidelines for the care of heart transplant recipients, coronary CT angiography (CCTA) is given a class IIb



recommendation (usefulness/efficacy is less well established by evidence/opinion) with a C level of evidence [2] [43, 44, 45].

CCTA shows promise in the evaluation of CAV in HTX recipients, although higher resting heart rates in these patients limit the technical image quality. It has got some limitations:

- high resting rates post-transplant
- radiation exposure
- contrast-induced nephropathy
- limited ability to assess smaller vessels

Cardiac CT imaging includes *coronary artery calcium (*CAC) CT scanning and CCTA. The following technical considerations are predominantly concerned with CCTA but are also relevant for understanding CAC CT scanning. Imaging the coronary arteries with CT is technically demanding.

Spatial and temporal resolution is challenged by the small, torturous vessels moving synchronously with the beating heart.

Electron beam CT was the first non-invasive imaging modality with cross-sectional visualization of the heart. It has a high temporal resolution of 100 ms, but the spatial resolution is limited by a slice thickness of 3 mm. With the introduction of multidetector technology, cardiac imaging with mechanical helical CT systems became possible. Starting out with cardiac imaging using 4-slice MDCT, a 64-MDCT is considered the minimum prerequisite for adequate scanning of the heart today *(see Figure 19)*.

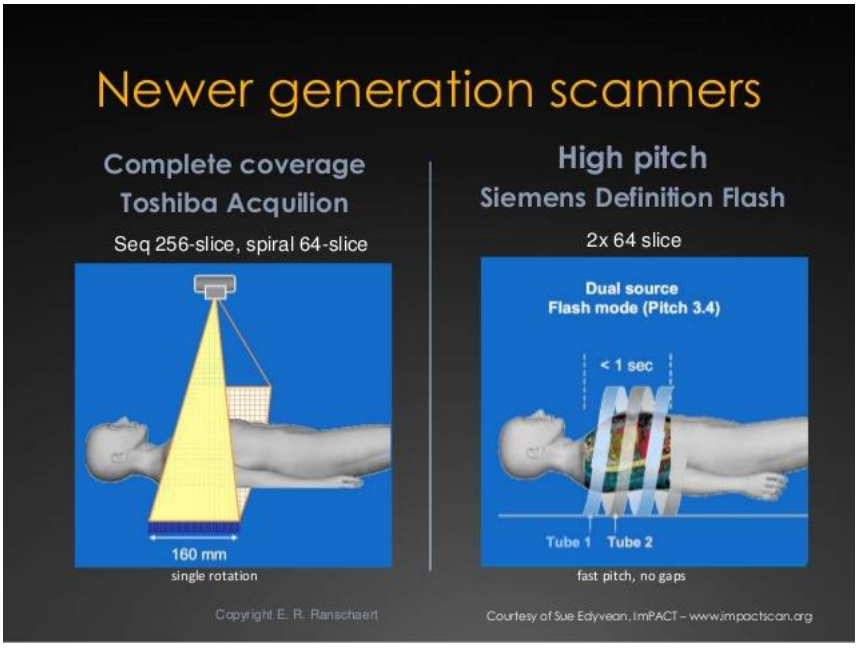


Figure 19: Images of the new generations scanner in cardiac computed tomography devices.

Spatial resolution

To be visualized adequately, the coronary arteries require isotropic submillimeter spatial resolution. Spatial resolution with contemporary 64-MDCT is 300–400 μm and is 230–240 μm on the newest high-end scanners (vendor website information; GE Revolution CT, Siemens Somatom Force). Coarse coronary calcifications are still a challenge to reliable visualization of the lumen because of blooming artifacts and reduce the specificity of CCTA.

Temporal resolution

High temporal resolution is a prerequisite for imaging the coronary arteries to avoid cardiac motion artifacts. The data acquisition time per image is referred to as temporal resolution. In cardiac imaging,

a half gantry rotation is sufficient for reconstruction of one image; therefore, temporal resolution is half the gantry rotation time. Dual-source (DS) systems with two x-ray tubes and corresponding detectors operating simultaneously provide temporal resolution close to a quarter of a rotation time, which presently is 66 ms with the fastest scanner (vendor website information; Siemens Somatom Force). Shorter rotation time enables adequate imaging of higher heart rates. Medication to lower the heart rate to 60–65 beats per minutes (bpm) is currently recommended by European guideline/American guideline. Another important temporal aspect is to minimize the time needed to cover the heart in the z-axis (the long axis of the patient). The optimum is to cover the heart in only one heartbeat to avoid misalignment artifacts related to the heart being differently positioned in consecutive heartbeats, which is especially noticeable in arrhythmia. One-heartbeat coverage is achieved with wide detector technology or with DSCT high-pitch technology. The widest detectors are 16 cm wide and cover the whole heart in one rotation. In high-pitch technology, the high pitch facilitates data acquisition of the whole length of the heart within the diastole of a heartbeat, and the dual detector system enables gapless volume coverage despite the high pitch by doing two helical acquisitions almost simultaneously.

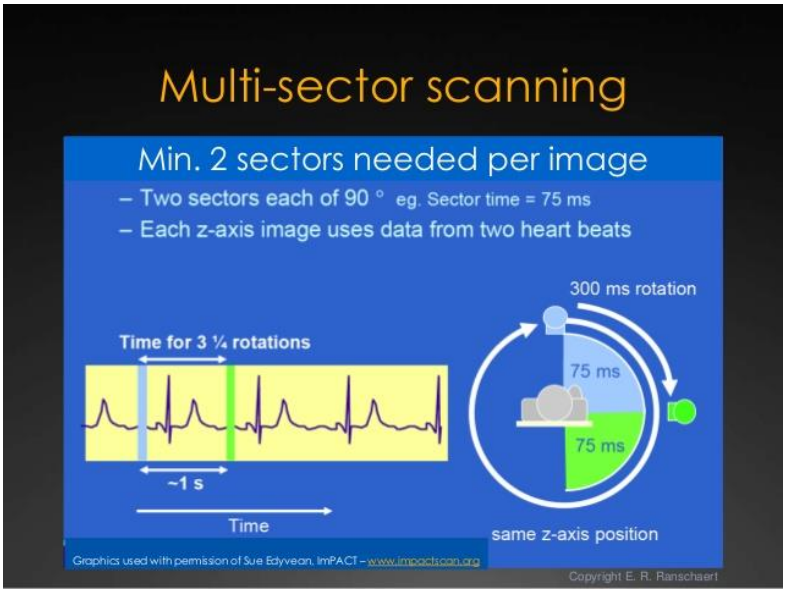


Figure 20: Multi-sector scanning and ECG

Scan modes

When imaging a beating heart, the images need to be reconstructed in consistency with a cardiac phase, i.e., systole or diastole. This reconstruction is facilitated with ECG-synchronized data acquisition. There are two types of ECG-synchronized scanning modes: retrospective ECG-gated helical scanning and prospective ECG-triggered axial (sequential) scanning. A variant of the prospective ECG-triggered method is used in high-pitch DS scanning where a helical data acquisition in the diastole of one heartbeat is prospectively triggered by the patient's ECG. In retrospective ECG-gating, the data are acquired in a continuous, helical scan and a continuous movement of the table

with simultaneous recording of the patient's ECG. The ECG recording guides data selection to ensure phase-consistent image reconstruction of data taken from several cardiac cycles. Sets of data can be reconstructed from any phase of the cardiac cycle, and the availability of both systolic and diastolic reconstructions makes the technique quite robust and can be essential in patients with high heart rates. In high heart rates, the optimal phase for reconstructing the left part of the coronary tree is most often the diastole while the right part is often best reconstructed in the late systole.

In prospective ECG-triggered sequential scanning, the data are acquired at a predefined phase of the cardiac cycle in an axial scan with a stationary table. Data acquisition is initiated by the patient's ECG signal using the R peak as a reference. Depending on the detector width, one or more sequential axial scans are needed to cover the entire heart volume. If more than one scan is needed, the table has to be moved to the next scan position between each data acquisition; hence, the term "*step-and-shoot*."

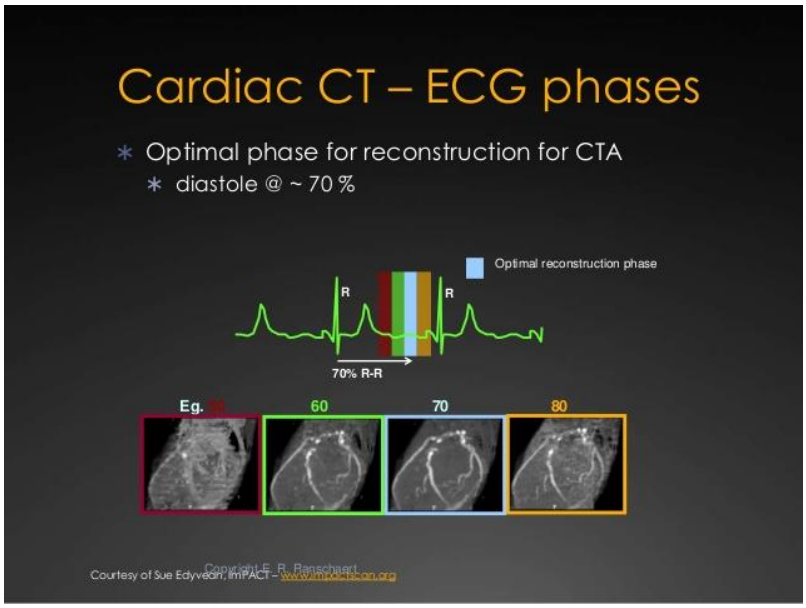


Figure 21: The ECG phases in CARDIAC CT scan

The prospectively ECG-triggered scan mode effectively reduces the time of radiation exposure because only a short part of the cardiac cycle is scanned. The short exposure time does, however, restrict the possibility of multiple reconstructions throughout the cardiac cycle. Prospective triggering is limited to patients with low heart rates (<70–75 bpm in systems with gantry rotation time 250–280 ms) and with stable sinus rhythm.

Radiation exposure

Dose-saving strategies: In all procedures involving ionizing radiation, the small stochastic risk of malignancy induction should be taken into consideration, and radiation exposure should always be kept as low as reasonably achievable. There has been much focus on radiation dose in cardiac imaging, and great efforts have been put into the development of dose-saving strategies by the vendors. The most important dose saving strategies/techniques are choice of scan mode, ECG-synchronized tube current modulation, tube voltage reduction, and iterative CT data reconstruction. Choice of scan mode is probably the single most important factor influencing radiation exposure. Prospectively ECG-triggered axial scanning significantly reduces the radiation dose compared to retrospective ECG-gated helical scanning; reductions of up to around 70–80% have been reported. In the latest generation of high-end scanners, submillisievert dose levels have been demonstrated with the combined use of ECG-triggered scan mode, lower tube voltage, automated exposure control, and iterative reconstruction algorithms with both high-pitch and wide-volume scanners.

Patient-related dose factors

Patient-related factors are important predictors of radiation dose. Heart rate and heart rate regularity are important determinants of radiation dose because most of the dose-reducing alternatives depend on a low and steady heart rate. Depending on gantry rotation speed, there is an upper limit for prospective ECG-triggered scanning of 60–65 bpm in earlier systems and 70–75 bpm in high-end scanners. Body weight is another factor with a profound effect on radiation dose Heavier patients require higher tube voltage and current to achieve acceptable image noise levels, which consequently increases radiation exposure.

Radiation dose parameters

The radiation dose parameters used for CT are volume CT dose index (CTDIvol), expressed in units of mGy, and dose-length product (DLP), expressed in units of mGy*cm. Simplified, CTDIvol is an estimate of the average radiation dose for a specific scan protocol for one tomographic image with pitch incorporated. DLP is the product of the CTDIvol and the scan length. The CTDIvol is recommended for optimizing CT protocols whereas DLP should be used for comparing radiation doses and characterizing radiation dose from CT studies. To estimate an effective dose for adult patients, the DLP is multiplied by an organ-weighting factor (k). In cardiovascular imaging, the k value for chest examination is used, which currently is 0.014 mSv per mGy*cm

3.3 Invasive methods

Invasive methods for coronary evaluation enclose: visualizing vessel lumen (coronary angiography), evaluation of vessel wall dimensions and wall components (IVUS, IVUS virtual histology, and optical coherence tomography [OCT]), and evaluation of coronary flow parameters (fractional flow reserve [FFR], and index of microcirculatory resistance (IMR)) [46, 47, 48, 49, 50].

Coronary Angiography (CA)

Although a relatively insensitive method for diagnosing CAV, CA remains the accepted standard of care serving as a screening tool to grossly detect the presence of CAV and is typically performed at an annual or biannual routine basis. The method is clinically available and has documented prognostic significance. The ISHLT recommendations for CAV nomenclature is based on angiographically depicted lesions of CAV and the surveillance recommendation is Class I [15, 2, 16, 51, 52]. It is prognostic for its accuracy in the detection of coronary stenosis and myocardial blush. Its limitations:

- evaluation limited to epicardial vessels
- insensitive for detection of early CAV
- insensitive for the detection of diffuse disease
- radiation exposure
- contrast-induced nephropathy.

The limitations are related to the nature of CAV lesions (concentric, longitudinal and diffuse disease) as well as expansive vascular remodeling.

Intra vascular Ultrasound (IVUS)

IVUS is superior to CA in detecting CAV. IVUS has been documented to detect CAV in apparently normal angiograms and to predict development of cardiac events even in the presence of a normal coronary angiogram [53, 15, 16, 45, 54]. A coronary artery intimal thickness ≥0.5 mm is defined as abnormal by ISHLT guidelines. A rapid progression of maximal intimal thickness (MIT) ≥0.5 mm during the first year after transplantation is a predictor of all-cause mortality and adverse cardiac events. On the other side, it has been demonstrated that IVUS-detected intimal hyperplasia does not correlate well with small-artery disease by histologic or immunohistochemical analysis.

Although IVUS is very sensitive for defining CAV, the ISHLT guidelines consider it to be an investigational tool and do not recommended IVUS for routine surveillance of CAV (Class IIa) [15] [16].

According to the same guidelines, IVUS is optional at baseline (5–6 weeks) and at 1 year after HTX to exclude donor CAD and detect rapidly progressive CAV, respectively, thus providing prognostic information. Being very sensitive for defining CAV, IVUS is an important research tool helping investigators to explore surrogate markers for CAV and evaluate the outcome of various therapeutic conditions.

Virtual histology (VH) is a relatively new IVUS-based technique providing information about plaque components. Four basic tissue components can be identified: fibrous, fibrofatty, calcified, and necrotic core. Although VH and IVUS are not a part of the routine surveillance of CAV, the added information on prevalence, morphologic patterns, and distribution from studies using these methods.

AUTHOR	JOURNAL	PARAMETERS
Park et al [55, 56]	JHLT 2017; 36: 185-192 EHJ 2016; 17: 272-279	•Vessel volume (mm3) •Minimal vessel diameter (mm) •Maximal vessel diameter (mm) •Lumen volume (mm3) •Minimal lumen diameter (mm) •Plaque Volume (mm3) •Percent Plaque volume
Clemmensen et al [57, 58]	JHLT 2016, 35 (4S): s98 JACC 2017, 10 (7): 773-784	• Plaque type • Mean Lumen/intima ratio • Maximal intima/media ratio • Percent plaque volume
Tomai et al [59]	JHLT 2016, 35 (1): 74-79	• EEL area (mm2) • IEL area (mm2) • Lumen area (mm2) • Plaque thickness (mm) • Media/EEL area (%) • Intima /media ratio • Intimal thickness (mm) • Type of plaque o eccentric o calcified o lipid pool
Khandar et al [60]	JHLT 2013, 32: 596-602	• Lumen area (mm2)

		• Intimal thickness (mm) • Media thickness (μm) • Intimal volume (mm3) • Media volume (mm3) • Plaque volume (mm3) • Plaque index % • I/M ratio

Table 3: the most important literature experiences of IVUS in the detection of CAV [58] [59] [56] [55] [60].

According to the scientific literature and experience (*see Table 3*), the IVUS most interesting data to analyze the CAV are:

- *Vessel volume (mm3)*
- *Minimal vessel diameter (mm)*
- *Maximal vessel diameter (mm)*
- *Lumen volume (mm3)*
- *Minimal lumen diameter (mm)*
- *Plaque Volume (mm3)*
- *Percent Plaque volume (%, following for the results)*

In particularly in the 2001, the American College of Cardiology Clinical Expert Consensus Document on Standards for Acquisition, Measurement and Reporting of Intravascular Ultrasound Studies [61] was published, which recommended a threshold for transplant vasculopathy as an intimal thickness of >0.5 mm measured at a target segment of a vessel. This is a widely accepted definition of CAV by IVUS today (see Table 4) [61].

	Class I	Class II	Class III	Class IV
Severity	Minimal	Mild	Moderated	Severe
Intimal thickness	<0.3 mm	<0.3 mm	0.3–0.5 mm	>1.0 mm
Extent of plaque	<180	>180	>0.5 mm, <180	>0.5 mm, >180

CAV, cardiac allograft vasculopathy; IVUS, intravascular ultrasound.
Reproduced from St Goar FG *et al.*[175]

Table 4: Stanford Classification of IVUS/CAV [61]

Optical Coherence Tomography (OCT)

OCT provides high resolution (10-20 μm) in the detection of microvascular disease and macrovascular epicardial disease. It is not included in the surveillance recommendation of the ISHLT. OCT gives many important CAV details such as arterial wall description, arterial lumen quality, plaque volume, plaque characterization. Its strengths are high spatial resolution and high accuracy in the description of the plaque and arterial lumen [62]. Its limitations are:

- evaluation is limited to epicardial vessels
- reduced tissue penetration than IVUS
- high cost
- limited availability

Invasive Coronary Flow Studies

The CAV causes complex changes in coronary physiology. Invasive coronary sensor pressure and flow wires allow independent assessment of the epicardial arteries and microvasculature by measuring the fractional flow reserve (FFR), coronary flow reserve (CFR), and index of microcirculatory resistance (IMR). At this time, it is not included in the ISHLT Surveillance Recommendation in the detection of CAV [16] [15] [58, 63]. There are some limitations:

- Cost
- Limited availability
- Risk of enhanced sensitivity to adenosine.

CHAPTER 4: Prognosis

The management of CAV is focused on primary prevention, early detection, imaging surveillance and early treatment as reported by Chih and other author in his recent review (*see Figure 22,23*) [35, 64, 65, 66]

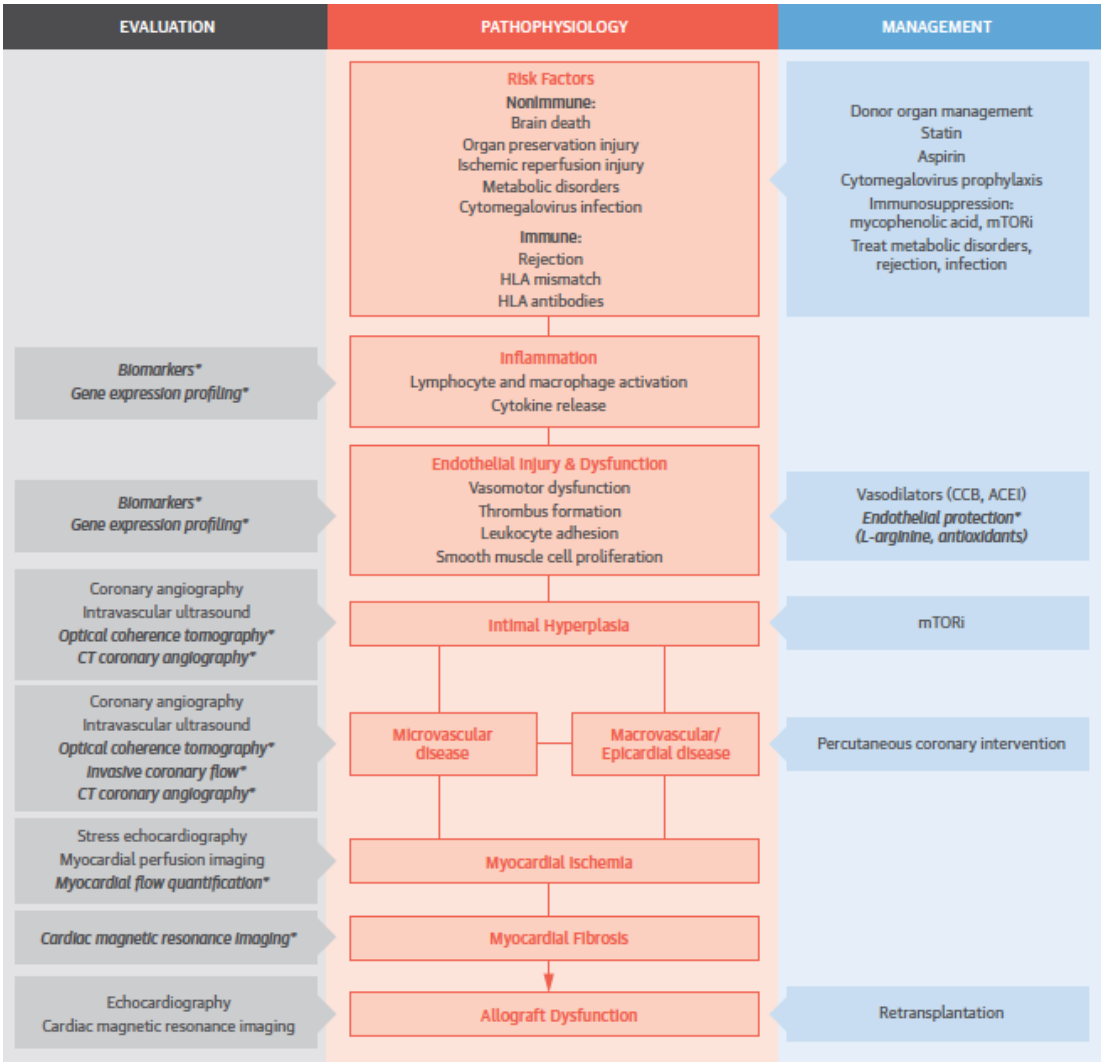


Figure 22: Algorithm for CAV surveillance and management.

In particularly the medications have a great role in the therapeutic choice.

- Aspirin: antiplatelet therapy; it reduces the formation of microthrombi at the sites of immune injury in the coronary endothelium [67];
- Statins: inhibit inflammatory and immune responses including the inhibition of natural killer cell cytotoxicity [68];
- Vasodilators: improved the microvascular function [35];

- Immunosuppression: mycophenolic acid reduces progression of intimal thickening, the mTORs sirolimus and everolimus inhibit vascular smooth muscle and fibroblast proliferation [69, 70, 71].

The Revascularization is limited to diffuse CAV and high mortality for surgical procedure.

First Author	N^ of pts	Procedural Success Rate	Adverse clinical outcome	Restenosis
Bader et al [72]	40	91%	20% 6 death 2 repeat OHT	BMS 31% DES 15%
Simpson et al [73]	33	99%	39,3%	6 mts: 31% 12 mts: 46% 5 years: 66%
Benza et al [74]	62	97%	34%	6 mts: 57%
Wellnhofer et al [75]	160	97%	/	38%
Lee et al [76]	82	100%	20%	DES 12% BMS 30%
Zakliczynski et al [77]	37	/	18%	DES 7% BMS 58%
Lee et al [78]	140	98%	25%	BMS 23% DES 10,4%
Tremmel et al [79]	34	/	12%	BMS 33% DES 12,5%

Table 5: The most important articles in scientific literature about the use of PCI in the treatment of CAV

The retransplantation is recommended to selected patients with advanced CAV but it is controversial because of organ storage, lower survival and re-presented CAV in de novo transplantation.

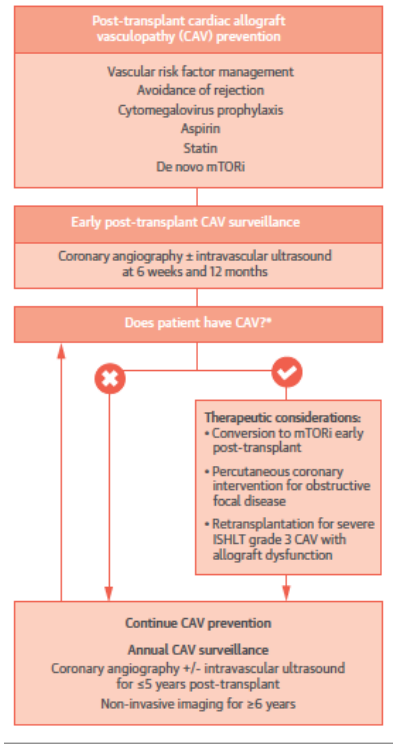


Figure 23: Preventive measures early post-transplant

CHAPTER 5: Study Design and Development

5.1 INTRODUCTION OF THE STUDY DESIGN

Firstly, I evaluate the capability of the 64-slice dual-source Coronary Computed Tomographic Angiography (CCTA) in the detection of Cardiac Allograft Vasculopathy (CAV) in the population of heart transplant recipients (***FIRST STEP***). In particularly I analyse the sensibility and specificity of CCTA versus the CA.

Then the CCTA is compared to intravascular ultrasound detection of CAV (IVUS, ***SECOND STEP***).

Finally, I compared the most important scientific articles regarding the early diagnosis of CAV with clinical, CCTA, CCA and IVUS and I create the "**CAV Early Diagnosis score (CAVeD score)**"

5.2 FIRST STEP

5.2.1 Material and Methods

Between January 2001 and December 2016, 84 patients undergoing heart transplantation at Heart Transplantation Center, Department of Heart and Vessels and followed by Heart Transplantation Ambulatory were screened for this retrospective observational study. Patients undergoing heart transplant in other Institution and subjects with renal failure were excluded from the analysis.

Data collection included patient demographics (age, sex, height, and weight), donor age, CAD risk factors (hypertension, diabetes mellitus, dyslipidemia, and current smoking history), dates of CCTA and CCA procedures, and current medications. Blood glucose, glomerular filtration rate according to MDRD (Modification of Diet in Renal Disease), and creatinine levels were also recorded. All data were prospectively collected and recorded onto computerized database registries that remained consistent over the study period. The study was approved by the Ethics Committee of our Institution. Human rights statements and informed consent: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later revisions. Informed consent was obtained from all patients for being included in the study.

Conventional coronary arteriography: Based on the ISHLT guidelines, CAV was classified by CCA as follows: CAV0 (not significant) indicates no detectable angiographic lesion; CAV1 (mild) indicates angiographic left main <50%, primary vessel with a maximum lesion of <70%, or any branch stenosis <70% (including diffuse narrowing) without allograft dysfunction; CAV2 (moderate) indicates angiographic left main <50%, a single primary vessel >70%, or isolated branch stenosis

>70% in branches of 2 systems without allograft dysfunction; and CAV3 (severe) indicates angiographic left main >50%, ≥2 primary vessels with >70% stenosis, isolated branch stenosis >70% in all 3 systems, or CAV1 or CAV2 with allograft dysfunction (defined as left ventricular ejection fraction <45%, usually in the presence of regional wall motion abnormalities).

Cardiac computed tomography angiography: The CCTA images were systematically analysed for image quality. Degree of CAV was assessed by using a 15-coronary segments model. The area under the receiver operating characteristic curve, sensitivity, specificity, and negative and positive predictive values of cardiac CT angiography for detection of CAV with any degree of stenosis and greater than or equal to 50%. CCTA was performed with CareDose, ECG pulsing MinDose to reduce radiation-dose and on retrospective cardiac synchronization (*see Table 6)*.

Table 6: Assessment of CCTA used to design the study

PARAMETERS	CHARACTERISTICS
Device	**Somatom Definition Siemens**
Sources	Straton tube focal spot oscillating (2 angled tubes 90 °)
Detectors	64x0,6 mm (FOV (50 cm) + 64x0,6 mm (FOV 25 cm)
Voxel	isotropic 0,4 mm
Tube-detector system rotation speed	330 msec
Temporal resolution	83 msec
Acquisition / cardiosynchronization mode	retrospective spiral
Pitch	0,2
Advance / Rotation	0,4 cm / rot
Advance / Second	1,2 cm / sec
kV / mA	120 kV / 400 mA
Radiant dose reduction technique (body)	Care Dose
Radiant dose reduction technique (cardiac)	ECG pulsing (min Dose)
Premedication	Natispray per os
Contrast Agent infusion	Contrast Agent 80 cc + Fisio 40 cc
Administration speed	5 cc / sec
Bolus timing	Bolus tracking (ROI in Asc Ao - limite 120 HU)
Trigger time - scan	7 sec
Reconstruction thickness	0,75 mm
Reconstruction intervals	0,5 mm
Filter	B26
Window	Mediastinum
Phases	Best Diastole + Best Systole + multifasic function (1mm 10-95% RR)

5.2.2. Statistical Analysis

Clinical data were prospectively recorded and tabulated with Microsoft Excel (Microsoft Corp, Redmond, Washington).

Continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test and compared between groups with unpaired Student t test for normally distributed values; otherwise, the Mann-Whitney U test was used. In case of dichotomous variables, group differences were examined by Pearson chi-square or Fisher exact tests as appropriate.

All variables subjected to univariate analysis and statistical value of $p < 0.05$ were further subjected to multivariate analysis (logistic regression).

For evaluation of diagnostic CCTA versus CCA, we calculated sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive (NPV) respectively [80]. These statistic parameters as well as disease prevalence and accuracy were expressed in percentages. The confidence intervals for Se, Sp and accuracy were exact Clopper- Pearson confidence intervals. The confidence intervals for predictive values were the standard logit confidence intervals given by Mercaldo et al (2007, [81]).

All analyzes were performed with Excel and Statplus 5.9 (AnalystSoft Inc., Walnut, CA).

5.2.3 Results

Data were analysed from 84 consecutive patients undergoing heart transplantation during a 204-month period from January 2001 to December 2016. Of these, 15 patients developed significant cardiac allograft vasculopathy (19,2% of the 84 consecutive patients). They formed the study population.

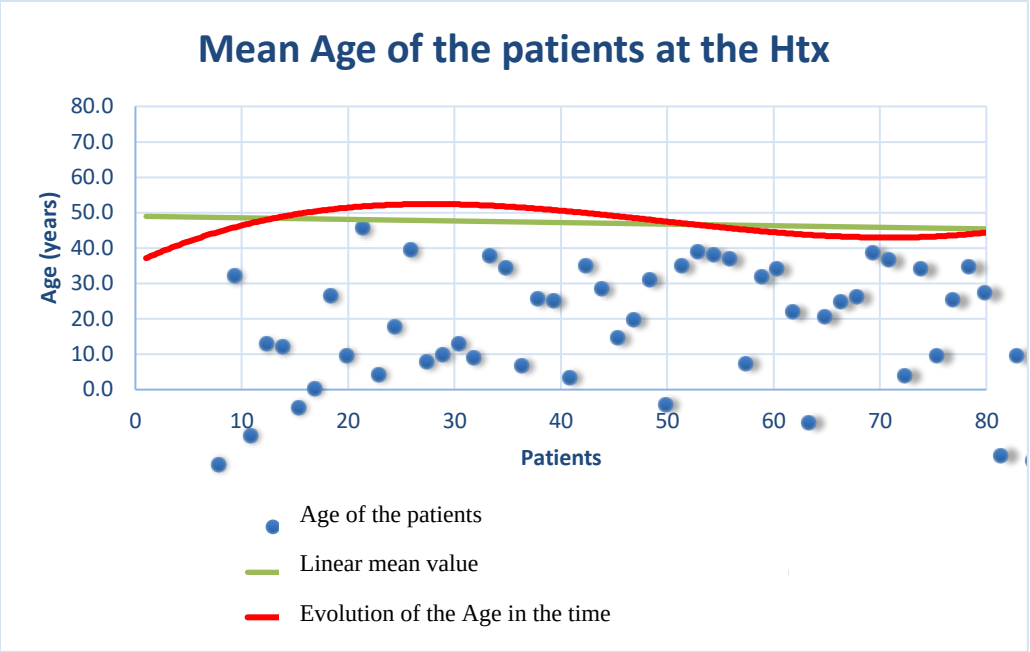


Table 7: Mean age of the patients at the heart transplantation surgery.

The 73,8% of candidate patients are male (n = 62) and the remaining 26,1% are female (n = 22).

The 34,88% (n = 30) of the population suffer from arterial hypertension, the 29,1% (n = 25) dyslipidemia, the 9,3% (n = 7) diabetes mellitus, the 13,95% (n = 12) chronic renal failure, and the 26,7% (n = 23) had previous coronary heart disease on native heart.

Of the study population, the critical cardiac allograft vasculopathy was developed in 15 patients (17.85%, Table 8).

	CAV	CAV %	NO CAV	NO CAV %	P Value
Gender Male	13	86,4	51	73,9	0,30
Gender Female	2	13,3	18	26,1	0,29
Mean Age (years)	45,9±10,8	/	47,6 ±11,5	/	0,62
Mean time from Htx (years)	4,2±3,6	/	7,9±2,7	/	< 0,0001
Arterial Blood Hypertension	7	33,3	23	33,3	0,33
Dyslipidemia	3	20	20	28,9	0,48
Renal dysfunction	3	20	12	17,4	0,81
Previous PCI	3	20	19	27,5	0,55
Diabetes	0	0	8	11,6	0,25

Crea (mg/dL)	1,22±0,59	/	1,4±0,5	/	0,03
Clereance Crea (ml/min)	74,4±22,8	/	72,2±21,1	/	0,71
Mean Ht (%)	37±4,6	/	33,7±8,7	/	0,15
Mean PCR (mg/L)	4,4±3,2	/	4,2±0,3	/	0,60

Table 8: Main differences between CAV group vs NO-CAV group. CAV: cardiac allograft vasculopathy, PCI percutaneous coronary interventions, PCR: C- reactive protein.

During the follow up, the patients underwent to CCTA, at a mean time of 3,8 months after heart transplantation, CCA, at a mean time of 1 months after heart transplantation.

We evaluated a total of 1260 coronary artery segments with a axial image reconstruction of 0,75 mm slice thickness.

The CAV was documented in 15 patients (17,8%) with at the mean age of 51±9,9 years from the heart transplantation, 110 ± 34 months after heart transplantation.

The CAV was documented as indicated in *Table 11/12.*

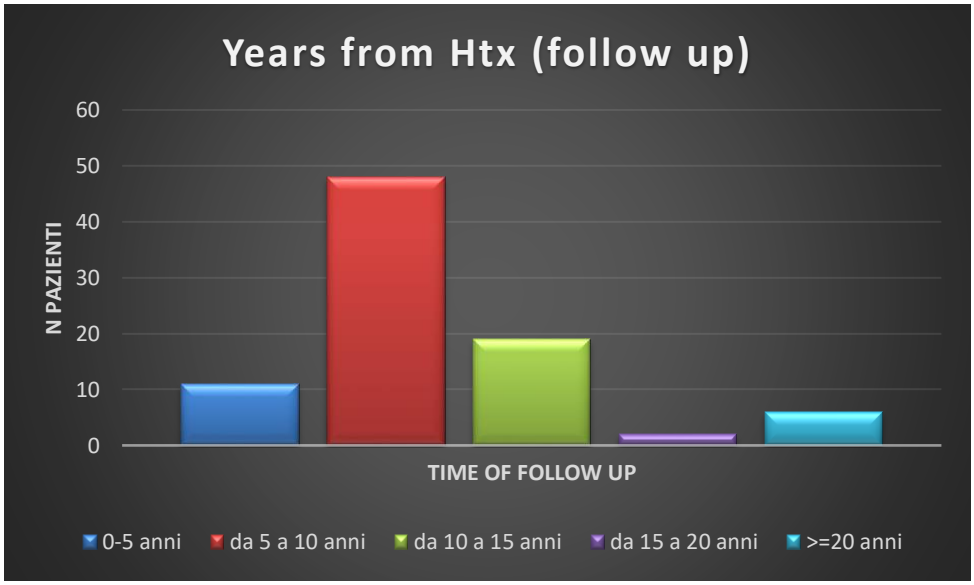


Table 9 : Years from heart transplantation (follow up).

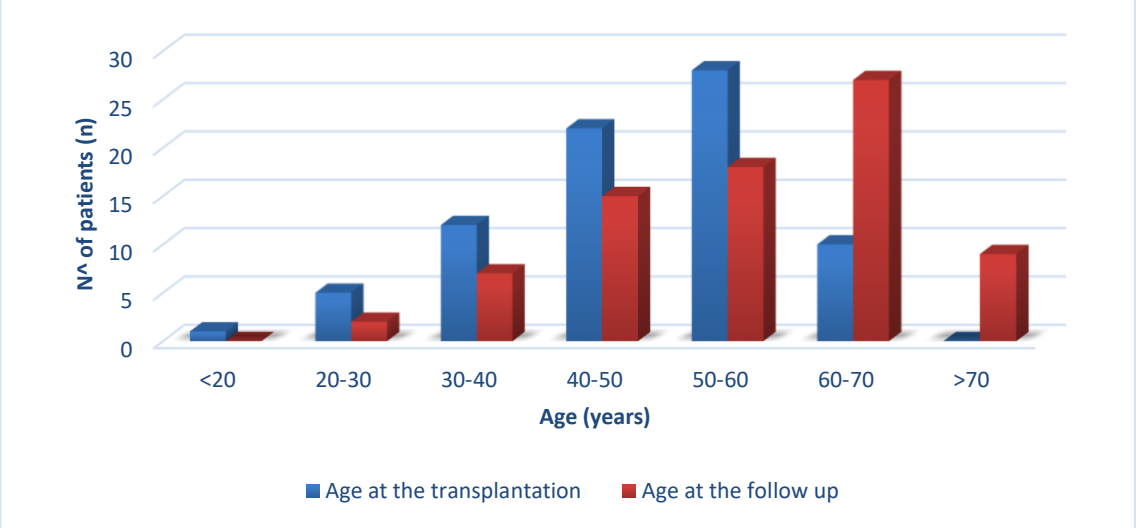


Table 10: the distribution of the patients according to the "Age at the Htx" and the "Age at the follow up".

The incidence of CAV was described in the *Figure 24*

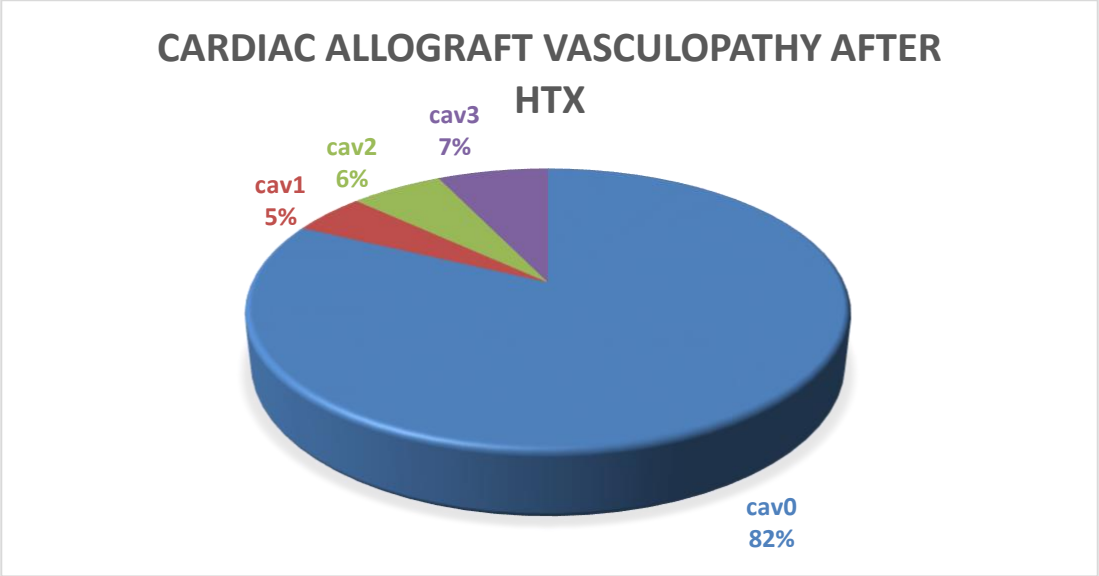


Figure 24: Incidence of CAV in the population

Considering the 1260 coronary artery segments studied of the CCTAs and the images of the CCAs, I calculated the sensitivities, specificities, negative predictive values and positive predictive values of CCTA versus CCA (*see Figure 25)*.

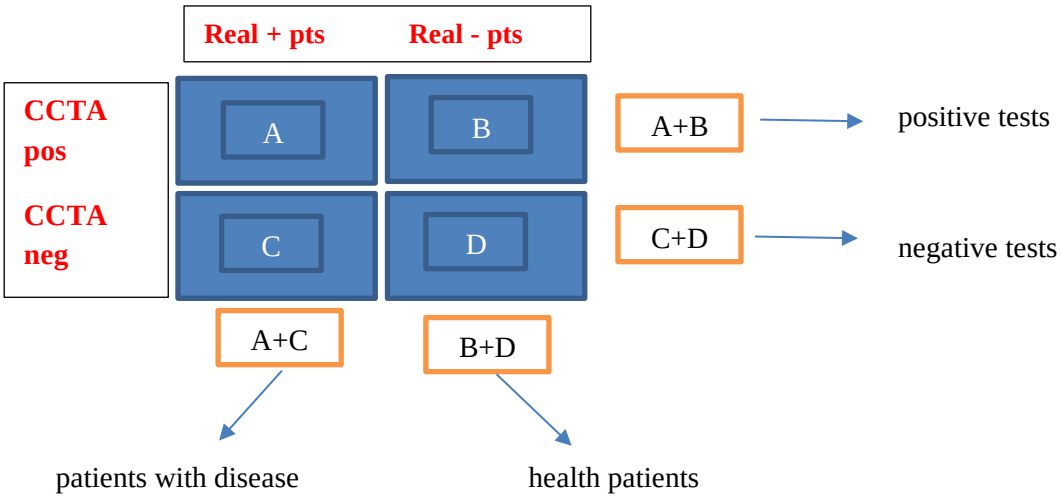


Figure 25: scheme of the calculation of the sensitivity, specificity

Considering a positive results of the tests when the grade of stenosis was equal or more of 50%, CCTA versus CCA showed mean sensitivities of 87,10% (95% CI 70,17%- 96,37%) and 97,22% (95% CI 85,47-99,93%), specificities of 99,84 (95% CI 99,41-99,98%) and 99,75% (95% CI 99,29-99,95%), a negative predictive value (NPV) of 99,68% (95% CI 99,19-99,87%) and 92,11% (95% CI 99,44-99,99%) and a positive predictive value of 93,10% (95% CI 77,07-98,19%) and 99,92%, (95% CI 79-97,31%) respectively (*see Figure 26-27*).

	Disease				
Test	**Present**	**n**	**Absent**	**n**	**Total**
Positive	True Positive	a=27	False Positive	c=2	a + c = **29**
Negative	False Negative	b=4	True Negative	d=1227	b + d = **1231**
Total		a + b = **31**		c + d = **1229**	

Figure 26: Baseline diagnostic test evaluator CCTA

	Disease				
Test	**Present**	**n**	**Absent**	**n**	**Total**
Positive	True Positive	a=35	False Positive	c=3	a + c = **38**
Negative	False Negative	b=1	True Negative	d=1221	b + d = **1222**
Total		a + b = **36**		c + d = **1224**	

Figure 27: Baseline diagnostic test evaluator CCA

The real prevalence of CCTA versus CCA was estimated as 2,46% (95% CI 1,68-3,47%) versus 2,86% (95% CI 2,01-3,93%), respectively.

The apparent prevalence of CCTA versus CCA was calculated to 0,0250 versus 0,0302.

The accuracy of CCTA versus CCA was 99,52% (95% CI 98,97-99,83%) versus 99,68% (95% CI 99,19-99,91%)

In addition, I evaluated the performance of the CCTA versus CCA directly. The “K” Cohen statistical parameter that documented the concordance and accuracy of the two diagnostical procedure was: 0,919 (range of optimal concordance, 0,81-1,00)

According to these great results of the high quality of CCTA in the detection of CAV versus CCA, I decided to improve the study with a second STEP.

5.3 SECOND STEP

5.3.1 Material and Methods

Between January 2001 and December 2018, patients undergoing heart transplantation at Heart Transplantation Center, Department of Heart and Vessels and followed by Heart Transplantation Ambulatory were screened for this observational study. Patients undergoing heart transplant in other Institution and subjects with renal failure were excluded from the analysis.

As the first step, the data collection included patient demographics (age, sex, height, and weight), donor age, CAD risk factors (hypertension, diabetes mellitus, dyslipidemia, and current smoking history), dates of CCTA and IVUS procedures, and current medications. Blood glucose, glomerular filtration rate according to MDRD (Modification of Diet in Renal Disease), and creatinine levels were also recorded. All data were prospectively collected and recorded onto computerized database registries that remained consistent over the study period.

The second step of the study was also approved by the Ethics Committee of our Institution. Human rights statements and informed consent: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later revisions. Informed consent was obtained from all patients for being included in the study.

Cardiac computed tomography angiography: The CCTA assessment was the same of the first step study to the new patients undergone to the protocol. The Table 6 reported the basic characteristics of the CCTA used for the study protocol.

Intravascular UltraSound Procedure (IVUS): I considered the following criteria to the diagnosis of CAV with IVUS by Minimal Lumen Area (MLA), according to the revision of the major experience of IVUS in scientific literature studies and the international guidelines.

Considering the MLA, I could detect three possible results:

- MLA < 6 mm $_2$: critical stenosis
- MLA 6-7.5 mm $_2$: requiring FFR procedure to calculate the stenosis
- MLA > 7.5 mm $_2$: “waiting and see”

In particularly, main criteria of IVUS diagnosis were explained in the following Figure 26. In case of small vessels (<3 mm), I considered the MLA significant when < 2 mm $_2$, plaque burden > 80%, LL> 20 mm and FFR < 0,75.

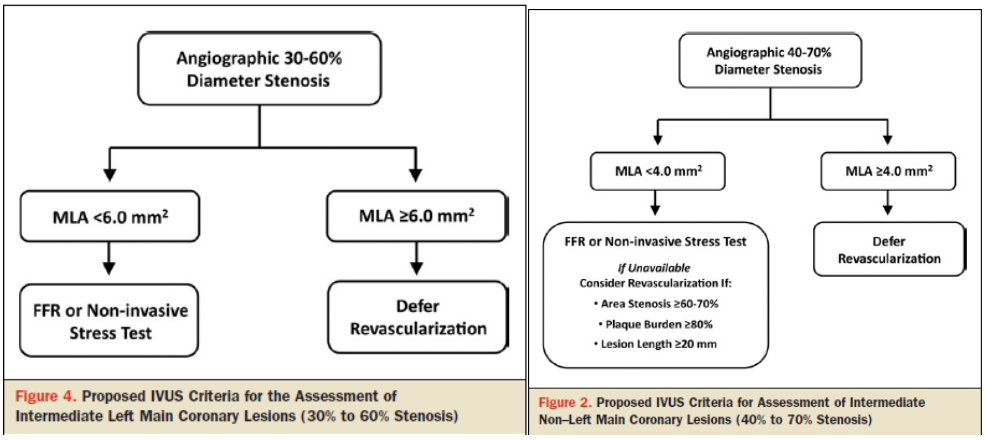


Figure 28: Criteria of diagnosis of coronary disease by IVUS in the Left Main coronary lesion and intermediate Non-Left Main coronary lesions.

Considering the Minimal Intimal Thickness (MIT), I supported the following classification:

- MIT < 0,3 mm: no disease
- MIT < 0,5 mm: see and follow
- MIT > 0,5 mm: index of CAV development

5.3.2. Statistical Analysis

Clinical data were prospectively recorded and tabulated with Microsoft Excel (Microsoft Corp, Redmond, Washington).

Continuous variables were tested for normal distribution by the Kolmogorov-Smirnov test and compared between groups with unpaired Student t test for normally distributed values; otherwise, the Mann-Whitney U test was used. In case of dichotomous variables, group differences were examined by Pearson chi-square or Fisher exact tests as appropriate. All variables subjected to univariate analysis and statistical value of p <0.10 were further subjected to multivariate analysis (logistic regression). For evaluation of diagnostic CCTA versus CCA, we calculated sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive (NPV) respectively [80]. These statistic parameters as well as disease prevalence and accuracy were expressed in percentages. The confidence intervals for Se, Sp and accuracy were exact Clopper- Pearson confidence intervals. The confidence intervals for predictive values were the standard logit confidence intervals given by Mercaldo et al (2007, [81]). All analyzes were performed with Excel and Statplus 5.9 (AnalystSoft Inc., Walnut, CA).

5.3.3 Results

Data were analysed from 93 consecutive patients undergoing heart transplantation during 216-months period from January 2001 to December 2018. Of these, 19 patients developed significant cardiac allograft vasculopathy (20,4% of the 93 consecutive patients). They formed the study population. Baseline demographic details were confirmed in *Table 11*.

PARAMETERS	VALUE
Age at the Htx (years)	47,9 ± 11,4
Gender Male (%)	68,8%
Age at the follow up (years)	56,1 ± 11,8
Arterial Blood Hypertension (%)	30%
Dyslipidemia (%)	25,8%
Chronic Renal Failure (%)	10,8%
Diabetes mellitus	12,9%
Previous AMI	30,1%
Previous PCI	23,6%
Previous CABG	20,4%
Cardiac Disease Familiarity	48,3%
Crea (mg/dL)	1,35 ± 0,49
GFR (ml/min)	72,7 ± 23
Ht (%)	36,6 ± 8,3
PCR (mg/dL)	7,2 ± 4,8
LVEF (%)	60 ± 8,8

Table 11: Baseline characteristics of the study population (CAV group and no CAV group). HtX: heart transplantation; AMI: acute myocardial infarction, Crea: creatinin, Ht: hematocrit, LVEF: left ventricle ejection fraction, PCR: C reactive protein, PCI: percutaneous coronary intervention, CABG: coronaric artery bypass graft.

Reviewing the update population, the critical cardiac allograft vasculopathy was developed in 19 patients (20,4 %). *See Table 12.*

	CAV	CAV %	NO CAV	NO CAV %	P Value
Gender Male	17	89,5	50	67,6	0,25
Gender Female	2	10,5	24	32,4	0,04
Mean Age (years)	46,5±9,9	/	47,6 ±11,5	/	0,71
Mean time from Htx (years)	4,5±3,6	/	7,7±2,6	/	>0,0001
Arterial Blood Hypertension	7	36,8	23	29,7	0,54
Dyslipidemia	5	26,3	20	23,8	0,81
Renal dysfunction	4	21,1	12	14,2	0,45
Previous AMI	7	36,8	26	30,9	0,62
Previous PCI	5	26,3	19	22,6	0,03
Previous CABG	2	10.5	0	0	0,002
Diabetes	2	10,5	9	10,7	0,97
Crea (mg/dL)	1,34±0,52	/	1,08±0,6	/	0,08
Clereance Crea (ml/min)	45,4±22,8	/	68,2±21,5	/	0,001
Mean Ht (%)	40,4±6,1	/	35,7±8,6	/	0,002
Mean PCR (mg/L)	4±3,29	/	4,2±0,3	/	0,57
LVEF (%)	60 ±5,8	/	61,8±6,9	/	0,29
Rejection	9	47,3	18	24,7	0,05
CD4/CD8 ratio > 2,5 postop	11	57,9	28	35,4	0,04

Table 12: Main differences between CAV group vs NO-CAV group. CAV: cardiac allograft vasculopathy, PCI percutaneous coronary interventions, PCR: C- reactive protein.

During the follow up, the patients underwent to CCTA, at a mean time of 3,1 months and IVUS at a mean time of mean time 9,5 months after heart transplantation.

The delay in the IVUS execution depended to hospital supplying, in the most of positive CCTA, the patient was undergone to IVUS, the interventional cardiologist was not directly informed to the CCTA results in order to not influenced the diagnostic value of IVUS.

We evaluated a total of 1395 coronary artery segments with an axial image reconstruction of 0,75 mm slice thickness and 18 IVUS procedures.

The CAV diagnosis according to ISHLT guideline was described in *Figure 29.*

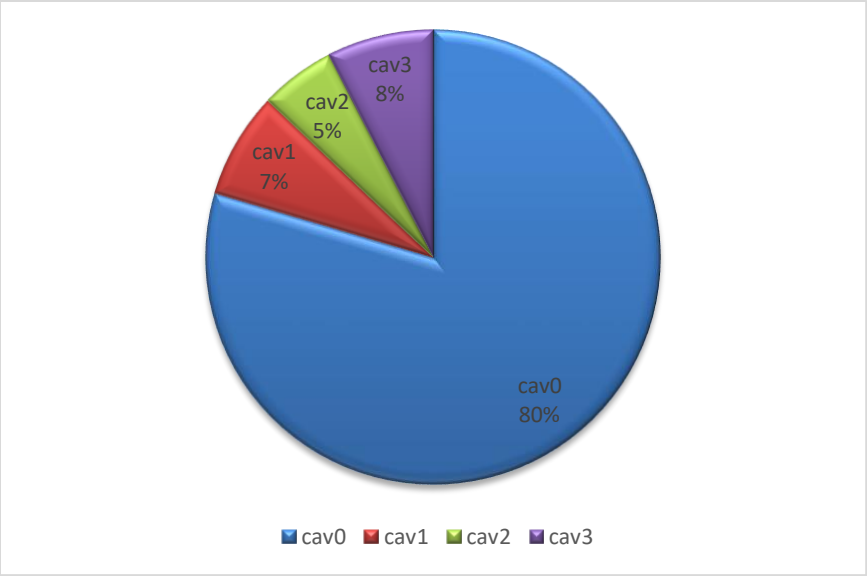


Figure 29: Incidence of CAV in the study population

The CAV in Htx patients were documented in following *Table 13.*

PARAMETERS	VALUE
Lesion location:	
a) Ostium	11
b) Bifurcation	8
Extent of disease vessel:	
a) left main	6
b) left main plus 1 vessel	2
c) left main plus 2 vessels	3
d) left main plus 3 vessels	1
e) 1 vessel without LM	6
f) 2 vessels without LM	2
g) 3 vessels without LM	0
Sites of stenosis:	
a) left main	12
b) descending anterior coronary	9
c) circumflex artery	4
d) right coronary artery	1
CCTA:	
a) CAV1	7
b) CAV2	5
c) CAV3	7
IVUS:	
a) MLA > 7,5	5
b) 7,5> MLA > 6	5
c) MLA < 6	9

Table 13: CAV characteristics in study protocol and their results.

Following the *Figure 30*, I calculated the mean value of sensitivities, specificities, NPV and PPV of the CCTA versus IVUS.

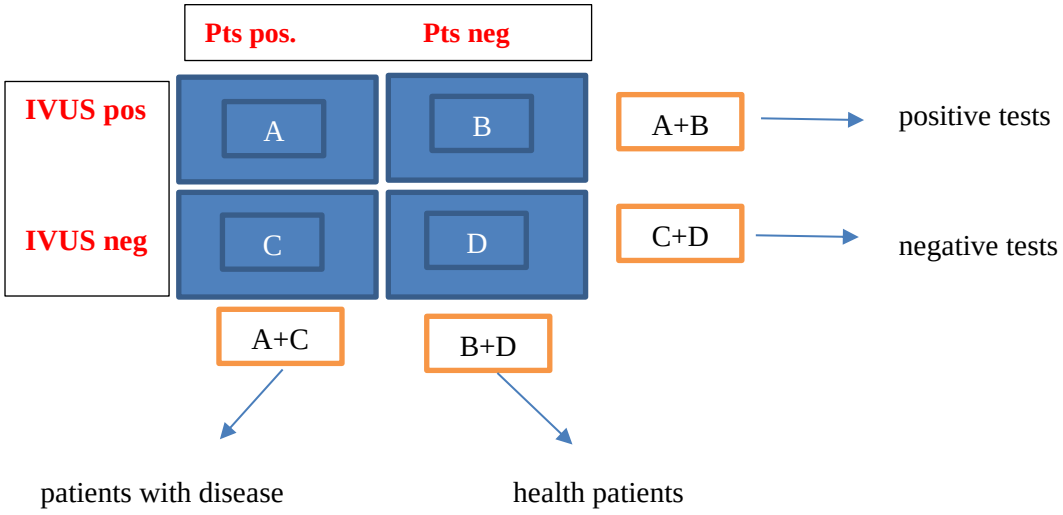


Figure 30: Schematic view of the calculation of sensibilities and specificities in CCTA versus IVUS

Considering a positive results of the tests when the grade of stenosis was with MLA < 6 mm2 and MIT > 0,5 mm, CCTA versus IVUS showed mean sensitivities of 94,74% (95% CI 82,25-99,36%) and 97,56% (95% CI 87,14- 99,94%), specificities of 99,71% (95% CI 99,25-99,92%) and 99,93% (95% CI 99,59-100%), a negative predictive value (NPV) of 99,85% (95% CI 99,43-99.96) and 99,49% (95% CI 99,48-99,98%), a positive predictive value of 90% (95% CI 77,13-96%) and 97,56% (95% CI 84,93-99,65).

The prevalence of CCTA versus IVUS was estimated as 2,72% (95% CI 1,93-3,72%) versus 2,94% (95% CI 2,12-3,97%), respectively.

The accuracy of CCTA versus IVUS was 99,57% (95% CI 99,07-99,84%) versus 99,86% (95% CI 99,48-99,98%)

At the end, considering the highlighted results in detection of CAV, CCTA versus CCA (First), CCTA versus IVUS (Second), I decided to improve the study with a third STEP: *a creation of CAV score to a potential early diagnosis of CAV in heart transplant recipient.*

5.4 THIRD STEP

5.4.1 Material and Methods

Baseline and operative characteristics of enrolled patients are summarized in Table 14-15.

5.4.2 Statistical Analysis

Considering the Fisher exact, Mann–Whitney, Kruskal–Wallis, and Cochran–Armitage tests were used for univariable analysis. Correlation between continuous variables was estimated by using the Spearman test. No was made attempt to replace missing values.

The dataset was randomly divided into a derivation dataset (75% of patients) and a validation dataset (25% of patients). Multivariable analysis of data from the derivation dataset was performed using stepwise logistic regression with backward selection.

The significance within the models was evaluated with the Wald test, whereas the strength of the association of variables with CAV was estimated by calculating the OR and 95% CIs.

Model discrimination was evaluated by using the area under the receiver operating characteristic (ROC) curve.

Only variables with a value of $P<0.05$ in univariable analysis were included in the regression models, to avoid overfitting (the use of regression in low population events may improve the accuracy and reduced inaccurate predictions of standard regression).

Furthermore, dichotomous variables with an OR <1.2 were excluded from the final regression analysis.

Additive risk score for the prediction of CAV in heart transplant patient (CAVeD score: Cardiac Allograft Vasculopathy early Diagnosis) was calculated by adding weighting points for each independent risk factor.

Once the predictive ability of the CAVeD score was tested in the validation dataset, further analyses were performed only in the overall dataset.

5.4.3: Results

Outcome of CAVeD SCORE: early diagnosis of cardiac allograft vasculopathy.

Predictors PARAMETRIC Variables (*see Table 14*):

PARAMETERS	SCORE 0	SCORE 1	SCORE 2	SCORE 3
AGE (years)	< 39	40-49	50-64	>65
Time from Htx (Years)	1-3	4-5	5-8	>8
Creatinine (md/dL)	< 1,2	1,2-2	>2	
ClCr (ml/m2)	>60	60-30	<30	
Hematocrit (%)	>46	45-40	39-30	< 29
LVEF (%)	>60	59-40	39-30	< 29
Rejection (n)	0-1	2-4	5-8	>8
CD4/CD8 ratio	<1.5	1,6-2	2,1-2,5	>2,6

Table 14: Predictors of parametric variables resulting from linear and multivariate regression analysis of patients data. HtX: heart transplantation, ClCr: clereance creatinine, LVEF: left ventricular ejection fraction, CD Cluster of differentations

Predictors NON Parametric Variables (*see Table 15*):

PARAMETERS	**SCORE 0**	**SCORE 1**
Gender Female	NO	YES
Previous CABG	NO	YES
Previous PCI	NO	YES

Table 15: Non-parametric predictors variables of the CAVeD score. CABG: coronary artery bypass graft, PCI percutaneous coronary interventions

Results of Multivariable Analysis for Prediction of CAV in the Derivation Cohort

Variables	**OR 95%**	**Coefficient**	**Additive Score Points**
Age			
40-59	2.04 (1.82 to 2.33)	0,717	1
60-64	2.73 (2.11 to 3,86)	1,078	2
>65	3.81 (3.32 to 4,32)	1,352	3
Gender Female	1.33 (1.14 to 1.65)	1,137	1
Time after Htx			
4-5	0,73 (0,45 to 1,19	0,815	1
6-8	1,11 (0,81 to 1,54)	0,967	2

>8	1,52 (1,29 to 2,8)	1,154	3
Previous PCI	1.93 (1.26 to 2.87)	0,654	1
Previous CABG	1.52 (1.17 to 1.89)	0,424	1
Crea			
1,3-2	1,49 (1,29vto 1,53)	1,112	1
>2,1	2,62 (229 to 3,09)	1,243	2
eGFR			
59-40	1,47 (1,01 to 2,14)	0,299	1
39-30	1,73 (1,18 to 2,52)	0,306	2
<29	3,23 (2,59 to 4,03)	1,283	3
Ht			
45-40	1,21 (0,51 to 2,89)	0,010	1
39-30	1,47 (1,18 to 1,83)	0,093	2
<29	1,48 (1,05 to 2,09)	1,081	3
LVEF			
59-40	0,77 (0,58 to 1,01)	0,325	1
39-30	0,86 (0,65 to 1,14)	0,582	2
< 29	1,19 (0,76 to 1,85)	0,681	3
Rejection			
2-4	1,05 (1 to 1,10)	0,572	1
5-8	1,28 (1,16 -1,42)	1,045	2
< 8	2,2 (1,67 to 3,16)	1,394	3
CD4/CD8 ratio			
1,6-2	1,09 (0,93 to 1,28)	0,167	1
2,1-2,5	1,11 (0,81 to 1,54)	0,568	2
>2,6	1,49 (1,29 to 1,73)	0,621	3

Table 16: Multivariate regression results of the parametric and non-parametric variables. This is approximative multivariate regression analysis: the statistical error is limited nut elevated due to small size of the populations and study groups. However, this is the proof to improve this study design in the performing of CAVeD score.

Risk Matrix Table (*see Table 17*):

	Unlikely	*Rare*	*Possible*	*Likely*	*Certain*
Insignificant	1	2	3	4	5
Minor	2	4	6	8	10
Moderate	3	6	9	12	15
Major	4	8	12	16	20
Catastrophy	5	10	15	20	25

Table 17: this is the simple risk matrix matching on severity and incidence of disease according to the scores

Probability of disease (see Table 18):

LEVEL	PROBABILITY	CAVed Score
Very low	< 1/100 (< 1%)	0-4
Low	1/10 (~10%)	5-9
Medium	1/5 (~20%)	10-12
High	1 / 2 (~50%)	13-20
Very High	< 1 / 2 (>50%)	21-25

Table 7: Probability of CAV according to CAVeD Score

CHAPTER 6: Discussion

Cardiac allograft vasculopathy is a disease of coronary artery typical in heart transplant recipient and the major long-term complication in the survival after 5-10 years the heart transplantation.

Firstly, I highlighted the relevance rule of CCTA in the early detection of CAV by comparing the CCTA with CCA in heart transplanted recipient, secondly the CCTA versus IVUS, and finally the creation of CAV Early Diagnosis Score (*see Figure 31)*.

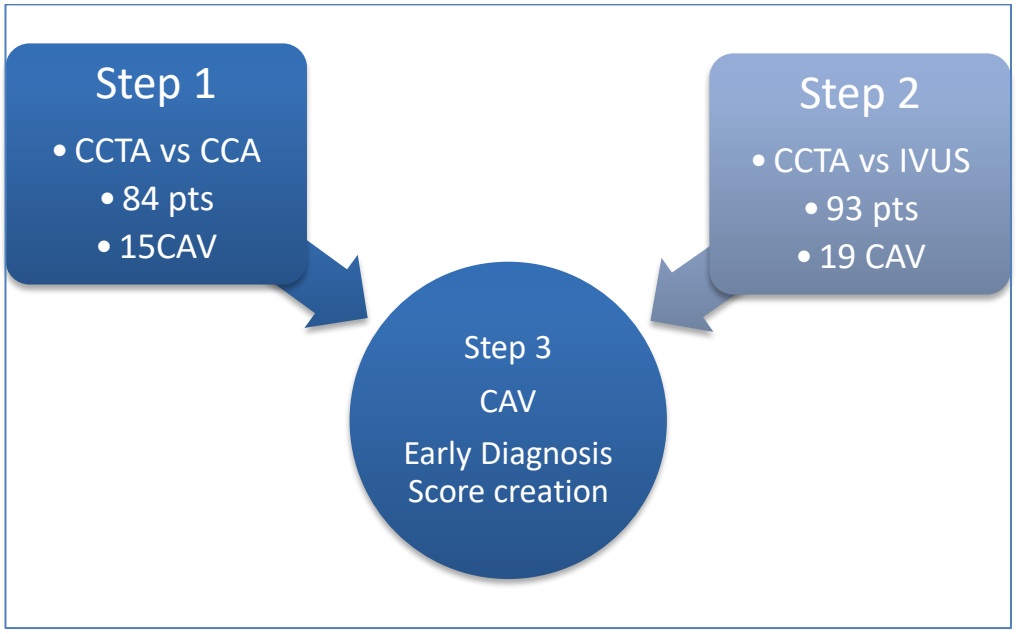


Figure 31: Study design and development

Although early survival after heart transplantation is limited by acute rejection, annual reports of the Registry of the International Society for Heart and Lung Transplantation (ISHLT) have suggested that CAV combined with late graft failure (likely because of allograft vasculopathy) accounted for 33% of deaths for those recipients who survived 5 years after transplant, followed in frequency by malignancies and non–cytomegalovirus transplantation, approximately 50% of recipients had angiographic evidence of CAV.

Our study attempts to provide a strategy by which to potentially reduce the incidence of CAV by easily and early detection. There are many observations to explain our findings.

The early diagnosis of CAV could contribute a prompt medical therapy hence increasing survival in heart transplanted patients. According to the literature I have reviewed the data about sensibility (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of the image procedure in the detection of CAV comparing to CCA.

Author	Year	Comparison	N^ of pts	Sensitivity	Specificity	NPV	PPV
Romeo et al [82]	2005	MDCT-16 vs CCA	50	80	99	80	99
Sigurdsson et al [83]	2006	MDCT-16 vs CCA	54	86	99	81	99
Iyengar et al [84]	2006	MDCT-16 vs CCA					
Pichler et al [85]	2008	MDCT-16 vs CCA	60	71	99	91	99
Schepis et al [86]	2009	DSCT vs CCA	30	93	80	48	98
Von Ziegler et al [87]	2009	MDCT-64 vs CCA	26	88	97	48	100
Nunoda et al [88]	2010	MDCT-64 vs CCA	22	90	97,5	81,8	98,7
Usta et al [89]	2009	MDCT-16 vs CCA					
Kepka et al [90]	2012	DSCT vs CCA	20	100	96,6	ND	ND
Barthelemy et al [91]	2012	MDCT-64/256 vs CCA	102	62,5	93,3	45,5	96,6
Mittal et al [92]	2013	MDCT-64 vs CCA	82	98	78	77	98
Wever-Pinzon et al [44]	2014	MDCT-64 vs CCA	203	97	81	78	97
Teudeberg et al [93]	2015	MDCT vs CCA		94	92		
Cottini et al [94]	2016	MDCT-64 vs CCA	11	99,2	99,5	86,6	98,1
Gunther et al [95]	2018	MDCT-64 vs CCA	41	100	98	7,7	100

Table 19: Scientific literature data about the sensibility, specificity, NPV and PPV in the comparison of CCTA to CCA.

CCTA is an easily available and reproducible diagnostic procedure that could help to detection of CAV with low costs and in the most of Radiology Departments.

Although the technical role of CCTA, the best way to increase survival of the Htx-patients is the follow-up: checking periodically patient and diagnosing possible disease and/or complication after heart transplantation is the first line in prevention. Considering the patient needs and the institutional limits, the follow up could be more specific and rapid for the patient but less expensive for the Institution. The CCTA satisfies these requests.

Moreover, a no-invasive and rapid exam could be more tolerate than others. The CCTA is no-invasive exam: a contrast material is injected by an automatic injection pump connected to the IV at a controlled rate. In addition, the CCTA was performed with Care Dose and ECG pulsing MinDose to reduce radiation-dose and on retrospective cardiac synchronization. These features allow high quality images with best spatial and contrast resolutions.

According to the study “FIRST STEP”, I have demonstrated acceptable results as rule out of CCTA versus CCA showed mean sensitivities of 87,10% (95% CI 70,17%- 96,37%) and 97,22% (95% CI 85,47-99,93%), specificities of 99,84 (95% CI 99,41-99,98%) and 99,75% (95% CI 99,29-99,95%),

a negative predictive value (NPV) of 99,68% (95% CI 99,19-99,87%) and 92,11% (95% CI 99,44-99,99%) and a positive predictive value of 93,10% (95% CI 77,07-98,19%) and 99,92%, (95% CI 79-97,31%) respectively (Table 20):

Diagnostic Procedure	Sensibility	Specificity	NPV	PPV
CCTA	87,1%	99,8%	99,7%	93,1%
CCA	97,2%	99,7%	92,1%	99,9%

Table 20: Results of the sensibility, specificity, NPV and PPV in the comparison of CCTA to CCA

Even if considering the limited of the small size of the population and the learning curve of the radiologists in the CCTA procedure, I had kept on to the second endpoint of my study.

The second step of the study was the comparison between CCTA and IVUS. Considering the ISHLT and the other heart failure and heart transplantation guidelines [2] [15], the invasive cardiac procedure like IVUS and OCT will be recommended in the diagnosis of cardiac allograft vasculopathy [16].

The scientific literature (Pubmed/*Medline)* showed the following data about the comparison of these two diagnostic procedures *(see Table 21*)

Author	Year	Comparison	N^ of pts	Sensitivity	Specificity	NPV	PPV
Gregory et al [96]	2006	MDCT-64 vs IVUS	20	70	92	89	77
Sigurdsson et al [83]	2006	MDCT-16 vs IVUS	13	96	88	80	98
Schepis et al [86]	2009	DSCT vs IVUS	30	85	84	76	91
Wever-Pinzon et al [44]	2014	MDCT-64 vs IVUS	12	81	75	50	93
Romero et al [97]	2014	MDCT-64 vs IVUS	615	81	75	93	50

Table 21: Scientific literature data about the sensibility, specificity, NPV and PPV in the comparison of CCTA to IVUS.

According to the international guidelines and recent original article/review and metanalysis, the invasive ultrasound technologies is a gold standard to detect the CAV but the evolution of CCTA software, spatial and temporal resolution has improved its quality and the diagnosis within to be competitive to IVUS *(see Table 22*). CCTA versus IVUS showed mean sensitivities of 94,74% (95% CI 82,25-99,36%) and 97,56% (95% CI 87,14- 99,94%), specificities of 99,71% (95% CI 99,25-99,92%) and 99,93% (95% CI 99,59-100%), a negative predictive value (NPV) of 99,85% (95% CI 99,43-99.96) and 99,49% (95% CI 99,48-99,98%), a positive predictive value of 90%

(95% CI 77,13-96%) and 97,56% (95% CI 84,93-99,65). The prevalence of CCTA versus CCA was estimated as 2,72% (95% CI 1,93-3,72%) versus 2,94% (95% CI 2,12-3,97%), respectively.

The accuracy of CCTA versus CCA was 99,57% (95% CI 99,07-99,84%) versus 99,86% (95% CI 99,48-99,98%)

Diagnostic Procedure	Sensibility	Specificity	NPV	PPV
CCTA	94,7%	99,71%	99,85%	90%
IVUS	97,6%	99,93%	99,49%	97,6%

Table 22: Results of the sensibility, specificity, NPV and PPV in the comparison of CCTA to IVUS

In particularly, I noticed the CCTA is a diagnostic procedure with:

1. *low cost*
2. *high availability on secondary and primary hospitals*
3. *high image re-construction quality*
4. *optimal negative predictive value*
5. *good ductility and maneuverability in the performance of diagnostic exam in patients with borderline value of renal kidney disease or tachycardia;*
6. *quick-execution*

Finally, the CAV Early Diagnosis was created.

The study had limits:

- small size
- high risk of standard error
- high risk of multivariate coefficient error
- limited experience
- learning curve of the radiologists for CCTA
- learning curve of cardiologists for IVUS
- limited system device of IVUS caused not completed (n=18, 94% of the CAV group)

Excluding all these factors, I calculated the multivariate analysis of significant value in the linear regression and I created the risk matrix table and the probability of disease according the scores (Table 20-21). I need to collect more and more data of heart transplant recipients to improve the score, but this basis could be the first line to create something much more.

In conclusion, even if the most of cardiologists discourage the use of CCTA in the detection of CAV, from my data the CCTA is completely comparable to CCA and IVUS in the early detection of CAV

and the CAVeD score could be an additional method to predict the risk of CAV in heart transplant patients according some predictors parameters.

CHAPTER 7: Conclusion

The conventional coronary angiography is actually the gold standard in the diagnosis and surveillance of cardiac allograft vasculopathy and the combination CCA with IVUS demonstrated the successful, excellent and specific detection of the disease. As the CCA, the optimal coherence tomography (OCT) is considered a sensible and specific intravascular imaging exam to detect CAV by the current literature opinions and the spreading use in all interventional cardiology department not only in the adult but children/young patients both.

Although the CCA/IVUS and OCT holding the roles of the best diagnostic exams for CAV, the continued technological advances associating with improvement of the non-invasive imaging could offer a new and powerful CCTA to assess the most of arterial wall and distal small vessel details but with requiring less contrast, radiation, cost and time.

The CCTA could represent a reproducible diagnostic imaging procedure in many Radiology Department on the country as documented in my study.

Considering the limits of population and technologies in many hospital, the evolution of CCTA software and the more and more confidence with it could be the most important details of the growing use of CCTA in heart transplant recipient to detect early diagnosis of CAV.

Finally, the CAVeD score could provide a first indirect evaluation of the risk of CAV in heart transplant patients and its development and growing by more data collection could improve its value in daily clinical practice and survival of heart transplant patiens.

Bibliography

[1] W. TJ, "Natural History of asyntomatic left ventricular systolic dysfunction in the community," *Circulation,* vol. 108, pp. 977-982, 2003.

[2] P. P., "2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution o," *European Heart Journal,* vol. 37, no. 27, pp. 2129-2200, 2016.

[3] B. E. G. A. e. a. Mozaffarian D, "American Heart Association Statistics Committee; Stroke Statistics Subcommittee. Heart Disease and Stroke Statistics-2016 Update: A report from the American Heart Association.," *Circulation,* vol. 133, p. e38–e360., 2016.

[4] F. C. M. T. e. a. Ceia F and E. Investigators, "Prevalence of chronic heart failure in Southwestern Europe: the EPICA study.," *Eur J Heart Fail ,* vol. 4, p. 531–9, 2002.

[5] A. J. G.-E. A. e. a. Gomez-Soto FM, "Incidence and mortality of heart failure: a community-based study.," *Int J Cardiol ,* vol. 151, pp. 40-5, 2011.

[6] M. R. F. J. e. a. Ohlmeier C, "Incidence, prevalence and 1-year all-cause mortality of heart failure in Germany: a study based on electronic healthcare data of more than six million persons.," *Clin Res Cardiol,* vol. 104, pp. 688-96, 2015.

[7] W. B. W. P. e. a. Zarrinkoub R, "The epidemiology of heart failure, based on data for 2.1 million inhabitants in Sweden.," *Eur J Heart Fail ,* vol. 15, pp. 995-1002, 2013.

[8] S. H. Sakata Y, "Epidemiology of heart failure in Asia.," *Circ J ,* vol. 77, pp. 2209-17, 2013.

[9] L. H. Lund, "Global Public Health Burden Of Heart Failure," *Cardiac Failure Review,* vol. 3, no. 1, pp. 7-11, 2017.

[10] C. M. N. M. e. a. Cheng RK, "Outcomes in patients with heart failure with preserved, borderline, and reduced ejection fraction in the Medicare population.," *Am Heart J ,* vol. 168, pp. 721-30, 2014.

[11] F. G. D. L. L. e. a. Gheorghiade M, "Congestion in acute heart failure syndromes: an essential target of evaluation and treatment.," *Am J Med,* vol. 119, p. S3–S10, 2006.

[12] T. J. L. D. e. a. Bhatia RS, "Outcome of heart failure with preserved ejection fraction in a population-based study.," *N Engl J Med ,* vol. 355, pp. 260-9, 2006.

[13] B. D. D. K. e. a. Nieminen MS, E. S. Investigators, H. F. Association and E. S. o. Cardiology., "EuroHeart Failure Survey II (EHFS II): a survey on hospitalized acute heart failure patients: description of population.," *Eur Heart J ,* vol. 27, p. 2725–36, 2006.

[14] M. J. B. B. Clyde W. Yancy, “2013 ACCF/AHA Guideline for the Management of Heart Failure,” *JACC,* vol. 62, no. 13, 2013.

[15] v. d. M. P. e. al, “ACC/AHA Versus ESC Guidelines on Heart Failure,” *Journal of the American College of Cardiology,* vol. 73, no. 21, pp. 2756-68, 2019.

[16] M. R. M. e. al, “International Society for Heart and Lung Transplantation working formulation of a standardized nomenclature for cardiac allograft vasculopathy—2010,” *J Heart Lung Transplant ,* vol. 29, p. 717–27, 2010.

[17] B. C. e. al, “Heart transplantation in patients seventy years of age and older: a comparative analysis of outcome,” *J Thorac Cardiovasc Surg,* vol. 121, pp. 532-41, 2001.

[18] J. Kobashigawa, “Report fromaconsensusconferenceon primary graftdysfunctionaftercardiac transplantation,” *J HeartLungTransplant,* vol. 33, p. 327–340, 2014.

[19] C. G. o. Christie JD, “A consensus statementoftheInternationalSocietyforHeartandLung Transplantation.,” *JHeartLungTransplant,* vol. 24, pp. 1454-9., 2005.

[20] E. K. Stehlik J, “The Registry of the International Society for Heart and Lung Transplantation:twenty- eighth adultheartransplantreport—2011.,” *JHeartLungTransplant,* vol. 30, pp. 1078-94, 2011.

[21] J. C. Roussel, “Outcome of Heart Transplants 15 to 20 Years Ago: Graft Survival, Post-transplant Morbidity, and Risk Factors for Mortality,” *J Heart Lung Transplant ,* vol. 27, p. 486–93, 2008.

[22] I. A, “Primary Graft Failure after Heart Transplantation,” *Hindawi Publishing Corporation Journal of Transplantation,* pp. 1-9, 2011.

[23] A. C, “Early graft failure after heart transplant: risk factors and implications for improved donor–recipient matching,” *Interactive CardioVascular and Thoracic Surgery ,* vol. 15, p. 57–62, 2012.

[24] P. D, “Primary graft failure after cardiac transplantation: prevalence, prognosis and risk factors,” *Interactive CardioVascular and Thoracic Surgery3,* no. 27, p. 765–772, 2018.

[25] A. J. A. G. G. T. R. S. S. A. e. a. Weiss ES, “Creation of a quantitative recipient risk index for mortality prediction after cardiac transplantation (IMPACT).,” *Ann Thorac Surg 2011,* vol. 92, pp. 914-21, 2011.

[26] P. V. I. M. M. Gupta D, “Effect of older donor age on risk for mortality after heart transplantation.,” *Ann Thorac Surg ,* vol. 78, p. 890–9., 2004.

[27] O. A. e. al, “Chronic renal failure after transplantation of a non renal organ,” *N Eng J Med,* vol. 349, pp. 931-40, 2003.

[28] A. E. S. J. Gao SZ, “Accelerated coronary vascular disease in the heart transplant patient: coronary arteriographic findings,” *J Am Coll Cardiol ,* vol. 12, pp. 334-40 , 1988.

[29] M. W. Daniel Schmauss, “Cardiac Allograft Vasculopathy Recent Developments3,” *Circulation,* vol. 117, pp. 2131-2141, 2008.

[30] H. K. J.-i. S. Mitsuaki Isobe, “T Cell Costimulation in the Development of Cardiac Allograft Vasculopathy,” *Arteriosclerosis, Thrombosis, and Vascular Biology,* vol. 26, p. 1447–1456, 2006.

[31] F. P, “Current perspectives on the immunopathogenesis of systemic sclerosis,” *ImmunoTargets and Therapy,* vol. 5, pp. 21-35, 2016.

[32] K. SJ, “Multimodality noninvasive imaging in the monitoring of pediatric heart transplantation,” *JASE,* vol. 30, no. 9, pp. 859-70, 2017.

[33] S. S, “Applicability of cardiogoniometry as a non-invasive screening tool for the detection of graft vasculopathy in heart transplantat recipient,” *Interactive Cardiovascular and Thoracic Surgery,* vol. 23, pp. 976-978, 2016.

[34] K. M, “Diagnosis of graft coronary artery disease,” *Curr Opin Cardiol,* vol. 22, pp. 139-45, 2007.

[35] S. Chih, “Allograft Vasculopathy,” *JACC,* vol. 68, pp. 80-91, 2016.

[36] “Follow-up of heart transplant recipients with serial echocardiographic coronary flow reserve and dobutamine stress echocardiography to detect cardiac allograft vasculopathy.,” *J Am Soc Echocardiogr ,* vol. 27, pp. 531-9, 2014.

[37] “Dobutamine stress echocardiography during follow-up surveillance in heart transplant patients: Diagnostic accuracy and predictors of outcomes.,” *Heart Lung Transplant ,* vol. 34, p. 710–7, 2015.

[38] O. M. K. D. Akosah KO, “Dobutamine stress echocardiography predicts cardiac events in heart transplant patients.,” *Circulation ,* vol. 94, pp. II283-8, 1996.

[39] v. D. R. V. P. Elhendy A, “Prediction of mortality in heart transplant recipients by stress technetium-99m tetrofosmin myocardial perfusion imaging.,” *Am J Cardiol ,* vol. 89, pp. 964-8, 2002.

[40] D. T. d. R. Mc Ardle BA, “Does rubidium-82 PET have superior accuracy to SPECT perfusion imaging for the diagnosis of obstructive coronary disease?: a systematic review and meta-analysis.,” *J Am Coll Cardiol ,* vol. 60, pp. 1828-37, 2012.

[41] L. D. B. S. Braggion-Santos MF, “Late gadolinium enhancement assessed by cardiac magnetic resonance imaging in heart transplant recipients with different stages of cardiac allograft vasculopathy,” *Eur Heart J Cardiovasc Imaging ,* vol. 15, p. 1125, 2015.

[42] S. J. N. J. Miller CA, “Multiparametric cardiovascular magnetic resonance assessment of cardiac allograft vasculopathy.,” *J Am Coll Cardiol ,* vol. 63, pp. 799-808, 2014.

[43] “2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes,” *European Heart Journal,* vol. 00, no. 171, p. 1□71, 2019.

[44] R. J. K. Wever-Pinzon O, “Coronary computed tomography angiography for the detection of cardiac allograft vasculopathy: a meta-analysis of prospective trials.,” *J Am Coll Cardiol ,* vol. 63, pp. 1992-2004, 2014.

[45] N. D. P. M. Costanzo MR, “Heart transplant coronary artery disease detected by coronary angiography: a multiinstitutional study of preoperative donor and recipient risk factors. Cardiac Transplant Research Database.,” *J Heart Lung Transplant,* vol. 17, pp. 744-753, 1998.

[46] P. F. L. N. Rickenbacher PR, “Prognostic importance of intimal thickness as measured by intracoronary ultrasound after cardiac transplantation,” *Circulation ,* vol. 92, pp. 3445-52, 1995.

[47] M. Y. H. J. Cassar A, “Coronary atherosclerosis with vulnerable plaque and complicated lesions in transplant recipients: new insight into cardiac allograft vasculopathy by optical coherence tomography.,” *Eur Heart J ,* vol. 34, pp. 2620-7, 2013.

[48] O. T. N. D. Ichibori Y, “Optical coherence tomography and intravascular ultrasound evaluation of cardiac allograft vasculopathy with and without intimal neovascularization.,” *Eur Heart J Cardiovasc Imaging ,* vol. 15, pp. 51-8, 2016.

[49] N. M. L. D. Fearon WF, “Simultaneous assessment of fractional and coronary flow reserves in cardiac transplant recipients: Physiologic Investigation for Transplant Arteriopathy (PITA Study).,” *Circulation ,* vol. 108, pp. 1605-10, 2003.

[50] S. O. Y. M. Watanabe T, “Donor-transmitted atherosclerosis associated with worsening cardiac allograft vasculopathy after heart transplantation: serial volumetric intravascular ultrasound analysis,” *Transplantation,* vol. 101, pp. 1310-1319, 2017.

[51] L. N. V. A. F. R. Jan M. Van Keer, “Long-term outcome of cardiac allograft vasculopathy: Importance of the International Society for Heart and Lung Transplantation angiographic grading scale,” *Journal of Heart and Lung Transplant,* p. article in press, 2019.

[52] K. C. W. C. D. Khush, “The international thoracic organ transplant registry of the international society for heart and lung transplantation: thirty-fifth adult heart transplantation report—2018; focus theme: multiorgan transplantation,” *J Heart Lung Transplant,* vol. 37, pp. 1155-1168, 2018.

[53] S. N. S. Z. K. Kapadia, “Impact of lipid abnormalities in development and progression of transplant coronary disease: a serial intravascular ultrasound study.,” *J Am Coll Cardiol,* vol. 38, pp. 206-213, 2001.

[54] S. P. A. K. S. Agarwal, “Long-term mortality after cardiac allograft vasculopathy: implications of percutaneous intervention,” *JACC Heart Fail. 2014,* vol. 2, pp. 281-288, 2014.

[55] T. S. Z. L. Kyoung-Ha Park, “Relationship between markers of plaque vulnerability in optical coherence tomography and atherosclerotic progression in adult patients with heart transplantation,” *JHLT,* vol. 36, pp. 185-192, 2017.

[56] T.-G. K. Y. M. Kyoung-Ha Park, “Association between the vasa vasorum and the atherosclerotic changes in cardiac allograft vasculopathy: volumetric analysis,” *European Heart Journal - Cardiovascular Imaging,* vol. 17, no. 3, p. 272–279, 2016.

[57] C. TS, “Incremental Value to Coronary Angiography by Optical Coherence Tomography for Detection and Interpretation of Cardiac Allograft Vasculopathy,” in *JHLT 2016,* Washingtown, 2016, 35 (4S): s98.

[58] H. N. E. H. Clemmensen TS, “Layered Fibrotic Plaques Are the Predominant Component in Cardiac Allograft Vasculopathy: Systematic Findings and Risk Stratification by OCT.,” *JACC,* vol. 10, no. 7, pp. 773-784, 2017.

[59] M. F. a. i. a. t. a. M. F. F. T. t. a. M. F. F. T. L. D. L. A. P. Fabrizio Tomai, “Optical coherence tomography for characterization of cardiac allograft vasculopathy in late survivors of pediatric heart transplantation,” *JHLT,* vol. 35, no. 1, pp. 74-79, 2016.

[60] S. J. K. H. Y. J. J. T. Sameer J. Khandhar, “Optical coherence tomography for characterization of cardiac allograft vasculopathy after heart transplantation (OCTCAV study),” *JHLT* , vol. 32, pp. 596-602, 2013.

[61] N. S. A. W. Mintz GS, “American College of Cardiology Clinical Expert Consensus Document on Standards for Acquisition, Measurement and Reporting of Intravascular Ultrasound Studies (IVUS). A report of the American College of Cardiology Task Force on Clinical Expert Consensus Do,” *J Am Coll Cardiol,* vol. 37, no. 5, pp. 1478-92, 2001.

[62] C. TS, “LAYERED FIBROTIC PLASQUES ARE THE PREDOMINANT COMPONENT IN CARDIAC ALLOGRAFT VASCULOPATHY: systematic and risk stratification by OCT,” *JACC Cardiovasc Imaging,* vol. 10, no. 7, pp. 773-84, 2017.

[63] D. A. Baran, “Shedding light on cardiac allograft vasculopathy,” *JACC Cardiovascular Imaging,* vol. 10, no. 7, pp. 785-786, 2017.

[64] T. S. O. I.-U. H. Sato, “Risk stratification for cardiac allograft vasculopathy in heart transplant recipients—annual intravascular ultrasound evaluation.,” *Circ J.* , vol. 80, pp. 395-403, 2016.

[65] E. L. N. K. M. T. M. S. J. Kransdorf, “Prediction model for cardiac allograft vasculopathy: comparison of three multivariable methods.,” *Clin Transpl,* vol. 31, 2017.

[66] A. V. H. H. S. Keogh, “Impact of proximal or midvessel discrete coronary artery stenoses on survival after heart transplantation.,” *J Heart Lung Transplant,* vol. 11, pp. 892-901, 1992.

[67] M. M. G. S. G. C. M. Kim, “Early Use of Antiplatelet Therapy and Development of Cardiac Allograft Vasculopathy: A 5 Year Outcomes Analysis,” *JHLT,* vol. 35, no. 4, p. S99, 2016.

[68] T. J. S. M. Harris J, “Optimal low-density lipoprotein concentration for cardiac allograft vasculopathy prevention,” *Clin Transplant,* vol. 32, no. 5, p. e13248., 2018.

[69] H. T. E. D. R. Eisen, “Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients.,” *N Engl J Med,* vol. 349, pp. 847-858, 2003.

[70] D. P. S. B. D. Mancini, “Use of rapamycin slows progression of cardiac transplantation vasculopathy.,” *Circulation,* vol. 108, pp. 48-53, 2003.

[71] J. D. D. V. C. O. Van Keer, “The CECARI study: everolimus (Certican(R)) initiation and calcineurin inhibitor withdrawal in maintenance heart transplant recipients with renal insufficiency: A multicenter, randomized trial.,” *J Transplant,* 2017.

[72] B. FM, “Percutaneous coronary interventions with stents in cardiac transplant recipients,” *J Heart Lung Transplant,* vol. 25, pp. 298-301, 2006.

[73] S. L, “Long-term results of angioplasty vs stenting in cardiac transplant recipients with allograft vasculopathy,” *J Heart Lung Transplant,* vol. 24, pp. 1211-7, 2005.

[74] B. RL, “Pallation of allograft vasculopathy with transluminal angioplasty: a decade of experience,” *J Am Coll Cardiol,* vol. 43, pp. 1973-81, 2004.

[75] W. E, “A decade of percutaneous coronary interventions in cardiac transplant recipients: a monocentric study in 160 patients,” *J Heart Lung Transplant,* vol. 27, pp. 17-25, 2008.

[76] L. S, “Comparison percutaneous coronary intervention with bare-metal and drug eluting stents for cardiac allograft vasculopathy,” *J Am Coll Cardiol Intv,* vol. 1, pp. 710-5, 2008.

[77] Z. M, “Comparison of long term results of drug-eluting stent and bare metal stent implantation in heart trasplant recipients with coronary artery disease,” *Transplant Proc,* vol. 39, pp. 2859-61, 2007.

[78] K. J. T. J. Lee MS, “Comparison of percutaneous coronary intervention with bare-metal and drug-eluting stents for cardiac allograft vasculopathy.,” *JACC Cardiovasc Interv,* vol. 1, no. 6, pp. 710-5, 2008.

[79] T. JA, “Comparison of drug-eluting versus bare metal stents in cardiac allograft vasculopathy,” *Am J Cardiol,* vol. 108, no. 5, pp. 665-8, 2011.

[80] M. D. B. T. G. M. Altman DS, Statistics with confidence, BMJ Books, 2000.

[81] L. K. Z. X. Mercaldo ND, Confidence intervals for predictive values with emphasis to case-control studies, 2007.

[82] Noworolski R et al, “Early and late indications for implantation of cardiac pacemaker in patients after heart transplantation a single center experience,” vol. 43, no. 8, 2011.

[83] D. TN, “Drug eluting versus bare metal stents in cardiac allograft vasculopathy: a systematic review of literature,” *Catheter Cardiovasc Interv,* vol. 1, no. 77, pp. 962-9, 2011.

Acknowledgements

During these three years, a lot of people contribute to my professional learning curve in surgery, clinical, statistic and personal skills improvements.

I would like to formally thank:

- Professor Francesco Musumeci, Dottor Mariano Feccia, Dottor Fabio Sbaraglia for their helps and support in the first experience in heart transplantation center;
- Dottor Maria Frigerio, for her support and teaching;
- Dottor Marco Lanfranconi and Dottor Aldo Cannata for their guidance and support in my experience in Niguarda Hospital;
- Dottor Tania Dominici, who gave the idea with the project started;
- Univesity of La Sapienza, The Staff and Professors of the Angio-Cardio-Thoracic Pathophysiology and Imaging Doctor of Philosophy (XXXII^Edition) and its coordinator Prof. G. Venuta

This project and my efforts would not have been successful without the support and help of a lot of colleagues, nurses and patients.

Finally, I would like to say special thanks to my husband Fabrizio, my family and my best friends for their support during the project and while writing up the thesis.