UNIVERSITÀ DI PARMA
UNIVERSITA' DI PARMA

Dottorato di ricerca in Scienze Mediche

Ciclo XXIX

STRESS-SCINTIGRAPHY VERSUS CONTRAST STRESS-ECHOCARDIOGRAPHY FOR PREDICTION OF HARD CARDIAC EVENTS

Coordinatore:
Chiar.mo Prof. Riccardo Bonadonna
Tutor:
Chiar.mo Prof. Riccardo Volpi

Dottorando: Dr. Carmine Siniscalchi

Dedico questa tesi a tutte le persone della mia vita,

alle persone che mi amano e che mi ameranno.

La dedico a tutti quelli che come me sognano.

Ai Maratoneti che ogni giorno corrono,

chilometro per chilometro,

fino alla meta.

Non la dedico ai qualunquisti,

a coloro che pensano con la testa di un altro.

Ricercatore significa mettere in dubbio,

Significa per questo essere libero,

libero di verificare, di dimostrare,

libero di non dimostrare, di sbagliare, di ricominciare.

Sono questo Ricercatore,

uomo libero prima che studente.

Sommario

1. ABSTRACT

Objectives: We compared the long-term prognosis of subjects without prior cardiac disease who underwent either vasodilator single-photon emission computed tomography (SPECT) or contrast stress-echocardiography (cSE) for suspected coronary artery disease (CAD).

Background: The routine use of ultrasound contrast media during cSE improves wall motion assessment and test feasibility, but the prognostic value of cSE in comparison with SPECT is not known.

Methods: Subjects who underwent vasodilator SPECT or cSE between 2008 and 2012 for suspected CAD but no history of cardiac disease were included. We compared the prognostic value of each method for combined all-cause death and non-fatal myocardial infarction, and their positive predictive value for angiographically obstructive CAD.

Results: 1387 subjects were selected, 497 who underwent SPECT and 890 cSE. During 4 years of mean follow-up there were 78 hard events in the cSE group and 51 in the SPECT group. Event-free survival in subjects testing positive for ischemia, either with SPECT or cSE, was significantly worse both in the overall population and after propensity-matching patients 1:1 for baseline characteristics ($p=0.032$ for SPECT and $p<0.001$ for cSE). In multivariable analyses (entire group or splitted SPECT or cSE subgroups) SPECT or cSE demonstrated significant stratification capability, an ischemic test doubling (SPECT) or more than doubling (cSE) the risk of future hard events independently from other variables. Positive predictive value of SPECT for the diagnosis of obstructive CAD was inferior to cSE, (PPV=63% vs 89% respectively, $p<0.001$).

Conclusions: Our study suggests that SPECT and cSE have comparable significant prognostic capability, with cSE demonstrating better diagnostic positive

predictive value for CAD. The absence of ionizing radiation and anticipated lower direct and also indirect costs from higher positive predictive value, suggest that cSE may be preferable to SPECT as a gatekeeper in subjects without a prior history of CAD.

2. ABBREVIATIONS

SPECT = stress single-photon emission tomography

CAD = coronary artery disease

SE = stress-echocardiography

cSE = contrast stress-echocardiography

MI = myocardial infarction

WM = wall motion

SDS = summed difference score

LVEF = left ventricular ejection fraction

PPV = positive predictive value

LAD = left anterior discending

CFVR = coronary flow velocity reserve

Keywords: scintigraphy; single photon emission computed tomography; contrast-echocardiography; stress-echocardiography; coronary artery disease; outcome; prognosis; positive predictive value.

3. INTRODUCTION

Stress single-photon emission tomography (SPECT) is still the most-commonly utilized imaging stress test in the suspicion of coronary artery disease (CAD) and for prognostic stratification. Several million SPECT studies are being performed each year in USA only, while stress-echocardiography (SE) today follows as the second most utilized imaging stress test. They are usually referred to as "functional testing" and they have been regarded as very accurate methods for the detection and risk stratification of CAD for decades. But their clinical use, effectiveness and feasibility may vary in the various centers. Although functional tests, such as SPECT and SE, are often treated as a single entity, they may well differ regarding their diagnostic sensitivity/specificity balance, overall accuracy, and also for their prognostic yield.

SPECT is also the single largest contributor to medical radiation burden and published estimates suggest that this burden may be causal of significant secondary disease in the community (1). The radiation dose of SPECT is about 15 mSv, depending upon type of technology, operator skills, and clinical questions. According to the updated risk estimates released in the Seventh Report of the authorative Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation (BEIR VII report), the attributable risk of cancer is 1/750 for 15 mSv exposure, corresponding to the dose estimate of a SPECT using 99mTc-Sestamibi tracer (2).

Stress-echocardiography (SE) is the second most common imaging stress test, implies no radiation burden and has low direct costs (less than half of SPECT), but its partly subjective interpretation, not infrequently due to suboptimal images, is perceived as a drawback. Both methods have been considered accurate for the detection and risk stratification of CAD for decades and both are deemed

appropriate for suspected CAD in symptomatic low, intermediate and high-risk subjects (3,4).

The true diagnostic accuracy of SE and SPECT for CAD has been questioned based on recent studies accounting for verification bias (5,6), which inflated sensitivity in previous-generation studies. Contrast stress-echocardiography (cSE) is able to improve the quality of wall motion (WM) assessment on which the method is based, thanks to endocardial border enhancement, leading to higher feasibility and reproducibility, potentially leading to higher accuracy (7,9). While exercise is the ideal stressor, a considerable number of imaging stress tests still need to be conducted using pharmacologic stressors (10). To date, no adequately-powered study has compared the long-term prognostic value of vasodilator SPECT versus cSE in a large population, although there are suggestions that cSE may be at least as effective (11). Comparison data may prove useful for the clinician to choose between SPECT and cSE for the large number of CAD-naïve patients presenting in current practice with suspect symptoms of CAD, since the radiation burden of SPECT would not be justified in case cSE is similarly accurate for risk stratification. We retrospectively evaluated all subjects without known CAD who underwent either vasodilator SPECT or cSE for suspected CAD in our centre, from 2008 (when we started to routinely use contrast during SE) through 2012. Our purpose was to assess their relative capability to stratify future myocardial infarction (MI) and all-cause mortality, and, secondarily to compare their positive predictive value for obstructive CAD in the subset of patients were indicated coronary angiography.

4. METHODS

4.1 Study Design And Population

Subjects were extracted from all consecutive patients recorded in the data banks of the University of Parma Medical Center, Parma, Italy, undergoing cSE or SPECT for suspicion of coronary artery disease or risk stratification from January 2008 to December 2012. Our tertiary centre is the only facility performing SPECT and SE studies in the greater Parma area, where approximately 450,000 people reside.

We excluded patients with either a history of prior MI, known CAD, significant congenital or acquired cardiomyopathy, severe valvular disease or other known co-morbidities reducing life expectancy to <1 year. The decision to choose either SPECT or cSE as the initial imaging provocative test for a specific patient was based on referring cardiologist preferences at the time of clinical visit, since the current analysis is retrospective, although databanks are prospectively collected. Because of the uncertain image quality at peak stress, all stress studies at our institution are performed with ultrasound contrast to guarantee optimal endocardial border enhancement, even when segmental wall motion is deemed interpretable at rest. This practice is also based on existing data suggesting that higher quality imaging is in any case a diagnostic advantage during SE (8-10).

Contraindications to indicate or perform SPECT or cSE were an unstable hemodynamic status or arrhythmias that require urgent evaluation, suspected acute coronary syndrome (by cardiac troponin and/or ECG data abnormalities), pregnancy or lactation, or known hypersensitivity to components of the stressors, radionuclide or contrast material utilized. Since dipyridamole was used as a pharmacologic stressor, patients with more than 1st degree A-V block or asthma were always excluded by pharmacologic tests. Available risk factors and

medication usage were recorded at the time of testing and pre-test probability of CAD calculated by the DICAD method (12). Hypercholesterolemia was defined as total cholesterol >200 mg/dL or treatment with lipid lowering medications. Hypertension was defined as blood pressure>140/90 mm Hg or use of antihypertensive medication. This study complies with the Declaration of Helsinki, and the research protocol was approved by the locally appointed ethics committee (Parma ethics committee, Italy).

4.2 Stress-echocardiography protocol.

Dipyridamole. Dipyridamole was always the stressor of choice and it was infused at the total dose of 0.84mg/kg in all patients. A total of 236 underwent a 10-minute 0.84mg/kg dipyridamole infusion+atropine administration (up to 1 mg), while the majority of patients (n=654) underwent a 6-minute protocol, consisting of the 0.84mg/kg dipyridamole infusion and no additional atropine administration. Two-dimensional echocardiography,12-lead electrocardiographic and blood pressure monitoring were performed in accordance to established standard protocols. Aminophylline was routinely used to reverse dipyridamole effect.

Ultrasound contrast protocol. The lipid-encapsulated microbubble SonoVue® (Bracco Imaging, Milan, Italy), was used as ultrasound contrast media, as repeated 0.5 ml bolus, for all studies at rest and peak stress. All cSE was performed using a commercially available ultrasound scanner, (iE33, Philips Medical Systems) equipped with low mechanical index real-time pulse sequence schemes. For this purpose the very-low mechanical index setting was set between 0.09 and 0.12, and frame rate between 24 to 40 Hz, depending on sector width/ depth. While myocardial perfusion was usually additionally assessed with flash-replenishment sequences in most cSE studies (13,14), in the current study such perfusion data were completely disregarded, and only the part of the final text report separately describing wall motion analysis was considered, while myocardial perfusion data in the report were not considered. Wall motion was assessed using the same real-time very-low mechanical index perfusion setting which was also utilized for perfusion assessement. There was no re-analysis of studies in the current study, only the text reports regarding wall motion data were

used. All studies were interpreted by reviewers who had performed and interpreted >500 cSE studies at our institution.

Image Analysis

The left ventricle was divided in 17 segments according to the recommendations of the American and European Societies of Echocardiography (15). Segmental WM was graded as follows: normal=1; hypokinetic=2; akinetic=3; and dyskinetic=4. Reversible ischemia was defined as the occurrence of a stress-induced new dyssynergy or worsening of rest hypokinesia in ≥1 segment. Inter-observer agreement data for WM assessment in our centre have been previously published (13,14).

4.3 Stress SPECT protocols.

99mTc-Sestamibi single-photon emission computed tomography was used. All included patients underwent pharmacological stress with dipyridamole 0.56mg/kg infusion for 4 min, because they reported or demonstrated inability to exercise maximally. Patients had to refrain from caffeine, methylxanthine-containing substances for at least 12 hours before testing. Stress and rest SPECT examinations were performed according to accepted guidelines, using the 17-segment left ventricle segmentation (16). Briefly, either one or 2 days protocol was used; a weight-adjusted dose of 99mTc-Sestamibi tracer (approximately 740 MBq + 740 MBq in 2 days protocol and 300 MBq + 900 MBq in one day protocol) was injected intravenously at rest and 2 minutes after the end of dipyridamole infusion. After a delay of 45–60 min (60 min delay for the rest phase) the ECG-gated stress images were acquired on a SPECT-CT Siemens Symbia T6 machine (dual-head

variable-angle gamma cameras with 6-slice diagnostic CT capability). Gated-SPECT was always used when possible.

Image Analysis

SPECT data were visually analyzed by an experienced nuclear medicine physician with more than 500 interpreted studies and who was experienced in the commercially-available quantitative Cedars QGS/QPS software package. All study classifications were semi-quantitatively analyzed as: no ischemia, mild, moderate, severe ischemia, based on the summed difference score (SDS), respectively of <2, 2-4, 5-8 or >8, as per guidelines (16-18).

A positive SPECT study for ischemia was defined in the current study using two different thresholds, either as the presence of any significant reversible perfusion defect (SDS >1) or, alternatively, as more often used in clinical practice, more-than-mild (SDS>4) reversible perfusion defect.

Follow-up. Data collection was performed through November 2016. Outcome was determined from patients' interviews at the outpatient clinic, hospital chart reviews, and telephone interviews with the patient, or referring physician. Primary outcome variables were death and non-fatal MI. To avoid misclassification of the cause of death, we considered overall mortality (19). Myocardial infarction was defined by typical symptoms, electrocardiographic, and cardiac enzyme changes. Follow-up time was considered starting from cSE or SPECT date until the first event or the last contact date. Follow-up data were analysed to evaluate event free survival according to cSE and SPECT result accounting for classical risk factors.

4.4 Study design and statistical analysis

This was an observational retrospective study of data collected in clinical practice. Given the retrospective nature of the study and for the purpose of minimizing the

bias in the comparison between the two methods, a propensity-matched subgroup of cSE and SPECT patients was identified according to baseline demographics (age and gender) and relevant clinical data (conventional risk factors and medications at the time of test) using a 1:1 ratio and the nearest neighbor method (20). Baseline characteristics were described as number of subjects and frequencies in case of categorical variables and considering mean and standard deviation for continuous variables. The distribution of variables between the cSE and SPECT groups were compared using independent sample T-test and $\chi 2$ test. A p value <0.05 was considered statistically significant.

A crude assessment of cSE and SPECT with respect to their ability to stratify the risk of future myocardial infarction and all-cause mortality was performed estimating event-free survival by tests result and comparing groups using the log-rank test.

Cox proportional hazards regression analyses were used to assess the relationships between tests result and risk of myocardial infarction and all-cause mortality.

Multivariable models were used to “control” for the effect of baseline patient differences. Variables whose P was <0.1 at univariable analysis were considered for the inclusion in multivariable model.

All analyses were performed both considering the initial group of patients (identified according to the pre-specified methods and criteria) and the propensity-matched subgroup.

In the subgroup of patients who underwent coronary angiography within 3 months after their test, the positive predictive value of cSE and SPECT was calculated and compared, with significant CAD defined as at least one visually-assessed coronary artery stenosis >50%.

5. RESULTS

The overall number of subjects who underwent cardiac stress cSE or SPECT *for suspicion of CAD* between 2008 and 2012 was respectively 2638 and 3021 studies, including both exercise and pharmacologic tests. After excluding tests using exercise stressor (18% of cSE and 69% of SPECT studies) and tests performed in patients with prior MI/known CAD or other exclusion criteria as per protocol in the current study, *890* patients with suspected CAD but no prior history of CAD were finally selected from the cSE database and *497* patients from the SPECT database. Therefore, the overall cohort of patients who underwent either stress test with vasodilator stressor and no exclusion criteria was finally composed of *n=1387* subjects. We both report baseline data in the overall population, and in the propensity-matched group.

Table 1 shows decriptive data for the cSE and SPECT groups, highlighting few significant differences ($p<0.05$) between cSE and SPECT populations, regarding anagraphical variables (age), prevalence of risk factors (smoke, hypercholesterolemia and hypertension) and medications at enrollment (aspirin and statins), while the pre-test probability of CAD was not different, and on average in the lower end of the CAD intermediate probability class (10%-90%) for both cSE (21%, 95% CI 11%-36%) and SPECT (22%, 95% CI 12%-39%).

Table 2 shows the same baseline variables after patients of the cSE group were matched (paired) with patients in the SPECT group, by so doing ending up with each group composed by 497 patients. After matching was applied, there were no significant differences between baseline variables of the two groups, making groups better comparable and baseline bias removed for further univariate and multivariable analyses. Only the percentage of abnormal SPECT (any degree of ischemia) was higher than abnormal cSE in this case (20.5% vs 13.8%, p=0.005),

while such percentages become similar (13.6% vs 13.8%, p=ns) when considering abnormal only more than mild ischemia at SPECT.

Outcome: During a median and mean follow-up respectively of 1628 days and 1515 days for the overall group of 1387 subjects, there were 139 hard events, 78 in the cSE group (8.8% of 890 cSE subjects), 37 non-fatal MI and 41 deaths, and 51 hard events in the SPECT group (10.3% of 497 SPECT subjects), 13 non-fatal MI and 38 deaths.

In the SPECT group 27 patients (5.4%), and in the cSE group 95 patients (10.7%) underwent revascularization within 3 months. No periprocedural MI was recorded.

After selecting only the 497 cSE subjects matched for baseline characteristics with the 497 SPECT patients, the number of events in the cSE was reduced to 46 hard events (9.3% of the 497 cSE subjects).

Event-free survival and event rates: Figure 1 Kaplan-Meier curves show the event-free survival for the outcome of myocardial infarction or death either in the overall groups of patients who underwent SPECT or cSE (left) and depending on SPECT and cSE results (right), either using crude data of the entire populations of n=890 cSE subjects and n=497 SPECT (upper panels) or matched populations of n=497 cSE subjects and n=497 SPECT subjects (lower). The definition applied here for a positive SPECT for ischemia is the presence of any degree of ischemia, based on a SDS >1 (including mild ischemia). In unmatched or matched populations, the event-free survival curves for positive or negative cSE or SPECT were significantly different (Log-rank for unmatched <0.001, matched Log-rank=0.002), although in the comparison between positive cSE vs positive SPECT, curves did not differ significantly (p=0.399 for comparison in unmatched, p=0.514 for matched).

Figure 2 shows Kaplan-Meier event-free survival curves for myocardial infarction or death depending on SPECT and cSE results, on the upper using crude unmatched data and on the lower after matching the two populations as previously described, but in this figure the threshold for a positive SPECT was set at the higher degree of SDS>4 (more than mild ischemia). Using this clinically widely used reporting for ischemia during SPECT (more than mild ischemia), it demonstrates better outcome stratification capability, compared with the definition used in Figure 1 of any form of ischemia (SDS>1). Applying the higher threshold definition for a positive SPECT, positive curve becomes now almost superimposable to the positive cSE curve, and the negative SPECT and negative cSE remain similar.

Figure 3 shows the event-rates at increasing follow-up time (years) from the index-test after matching the cSE and SPECT groups, as previously described. The outcome of myocardial infarction or death is significantly stratified by both cSE and SPECT ($p<0.001$ and $p=0.032$, respectively). The definition applied here for an ischemic SPECT is again based on more than mild ischemia, a SDS>4. The yearly hard event-rate for negative cSE and SPECT is similarly around 1.5-2%, while a positive test demonstrated more than double that yearly event-rate, with an apparent worse outcome for a positive cSE compared with SPECT, at least in the first few years, whereas later there is a catchup phenomenon with proportionally reduced difference in outcomes between subjects with abnormal SPECT and abnormal cSE, overall showing no significant differences.

Univariable and multivariable analyses: The univariable Cox proportional hazard model for prognostication of outcome in the overall 1387 cohort of subjects who underwent either SPECT or cSE is shown in Table 3. Age, male gender, hypertension, diabetes, aspirin, clopidogrel and left ventricular ejection fraction

(LVEF) <50% considered as single variables all increase the risk of events. An abnormal dipyridamole SPECT or cSE (compared with negative cSE as a reference) also increased the risk of events (abnormal SPECT HR 2.78 95%CI 1.47-5.25, cSE HR 3.83 95%CI 2.43-6.03).

Table 4 shows the univariable Cox proportional hazard model in the matched-cohort including overall n=994 1:1 matched subjects. The results are similar to table 3, with the exception that in this case clopidogrel results not statistically significant (p=0.083). The multivariable Cox proportional hazard model for prognostication of hard events confirms a risk-increasing role for age, male gender and diabetes. LVEF<50%, positive SPECT and positive cSE were still independently predictors of risk. However an abnormal SPECT vs normal cSE had only borderline significance (p=0.051) (abnormal SPECT HR 1.95 95%CI 0.998-3.81, cSE HR 1.98 95%CI 1.047-3.75).

Table 5 shows multivariable Cox proportional hazard model separately performed in SPECT and cSE groups after 1:1 propensity-matching as previously described. Not differently from the multivarable analysis in the overall group, SPECT and cSE demonstrated a significant stratification capability, doubling (SPECT) or even more than doubling (cSE) the risk of future hard events independently from demographic and clinical variables.

Coronary angiography and positive predictive value: When compared to angiography, positive predictive value (PPV) of SPECT was also significantly inferior to cSE, with only 22 out of 40 patients who underwent coronary angiogram because of positive SPECT truly demonstrating obstructive CAD (PPV=55%) when considering any ischemia at SPECT (SDS>1), and 19 out of 30 (PPV=63%) when using the definition of more than mild ischemia (SDS>4). For cSE 87 out if 98 demonstrated obstructive CAD (PPV=89%) which was proportionately higher

than either interpretative PPV for an ischemic SPECT ($p<0.001$ and $p=0.001$ respectively).

Side effects: During or after the tests there were no severe or life-threatening events. Temporary headache was more often recorded (39% vs 14%, $p<0.001$) associated with the higher-dose dipyridamole infusion (0.84mg/kg) used during cSE, compared with the lower-dose infusion during SPECT (0.56mg/kg). Headache always resolved after aminophylline infusion.

6. DISCUSSION

Our study is the first clinical comparison between cSE and SPECT regarding their long-term prognostic value in propensity-matched patients. It suggests that the two most frequently worldwide-utilized types of vasodilator imaging stress tests, namely SPECT and SE (this latter “upgraded” in the current study using contrast for best image quality) are capable of predicting myocardial infarction or death.

Our study specifically applies to patients referred for suspicion of CAD without a prior diagnosis of CAD. cSE was instead better when it comes to diagnostic positive predictive value for obstructive CAD in the subgroup undergoing coronary angiography, indicating that an abnormal SPECT study may be less specific for detecting obstructive CAD. This finding has been demonstrated by others (7), and suggests that the overall prognostic and diagnostic profile of cSE would positively impact downstream financial costs without affecting patient outcome.

Another point is the role of radiation risk of SPECT versus cSE.

The radiation dose of SPECT is about 15 mSv, depending upon type of technology, operator skills, and clinical questions. According to the updated risk estimates released in the Seventh Report of the authorative Committee to Assess Health Risks from Exposure to Low Levels of Ionizing Radiation (BEIR VII report), the attributable risk of cancer is 1/750 for 15 mSv exposure, corresponding to the dose estimate of a SPECT using 99mTc-Sestamibi tracer (2).

For this reason, considering the absence of ionizing radiation and hence the lower biological and environmental impact (21,22) lead us to propose that cSE should be preferred over SPECT in subjects with symptoms of suspected CAD and no prior cardiac disease.

On the other hand, patients with previous CAD were per-protocol excluded from this study, and it is unclear if these differences in PPV would apply, and what effect negative and positive studies have on outcome. cSE might be less accurate in patients with baseline wall motion abnormalities, while SPECT could theoretically be superior because of its inherent ability to assess for viability in this setting.

cSE is a form of technically optimized SE, associated with higher feasibility, reproducibility and, possibly, accuracy compared with standard SE. In the current study we purposefully refrained from incorporating cSE myocardial perfusion assessment (and/or Doppler coronary flow reserve on the left anterior descending coronary artery) potentially available during cSE, which would possibly add prognostic accuracy and diagnostic sensitivity to the test interpretation based only on wall motion (11,13,14), but adding complexity to the examination, perceived as too technically demanding to become truly clinically widespread; contrast endocardial border enhancement is instead very simple and can be easily applied in every day clinical practice, as long as presets are used to lower the mechanical index and allow complete left ventricular cavity opacification.

Patients with no prior history of CAD but a suspicion for CAD symptoms and a normal imaging stress test are expected to demonstrate very low combined event-rate (<1% cardiac mortality+non-fatal MI /year) and this may appear lower than the 5-6% hard events reported in the current study at 3-year follow-up; it should be stressed that we here reported *total mortality* by any cause in our “death+non-fatal MI” combined endpoint, the incidence of *total* mortality being approximately 3 times that of *cardiac* mortality (23), so that, after adapting our data to cardiac mortality, as mostly reported in prior SPECT or SE studies, current results are in line with previous literature (24).

Although the study population ranged at the lower-end of the intermediate CAD risk class, an abnormal result turned out to be predictive of a worse outcome, with approximately 15% (SPECT) to 20% (cSE) hard events at 3 years. Patients referred for abnormal dipyridamole SPECT or cSE studies and no prior known CAD are clearly at high risk of events and in need of aggressive medical and possibly interventional therapy.

No direct comparison between cardiac magnetic resonance and cSE is available in the literature and, as far as it is possible to compare different studies, in a recent summary describing overall data from stress cardiac magnetic resonance risk-stratification studies (25), patients without previously known CAD are credited with yearly 3% all-cause mortality if no ischemia at first-pass perfusion, while yearly 6.3% incidence if ischemia is detected: such data does not appear to better stratify risk compared with cSE results in our current study. However, patients referred for stress MRI may be at higher pre-test risk, and propensity matching would be required for any true comparisons.

6.1 *Study Limitations*

The current results need to be interpreted with caution due to the retrospective and single-centre design. Nonetheless, there are apparently no prior studies in the literature comparing vasodilator SE, performed with contrast for endocardial border enhancement, with vasodilator SPECT in suspected CAD patients, and the long follow-up period recorded also confers adequate statistical power, since we anticipated a low event-rate in such type of patients without known CAD. The single-centre nature of the study, considering the retrospective design, is probably not a drawback in this case, since a superimposable geographical, genetic, racial, risk factors and age distribution is expected in a single centre for both SPECT and cSE patients.

In the current study we preferred not to censor patients undergoing revascularization from follow-up, considering that introducing the bias of a possible confounding effect of coronary revascularization on outcome (26, 27) was less for a comparison study, and any revascularization bias would similarly affect both SPECT and cSE subgroups.

Enrolled pharmacologic cSE patients were more than pharmacologic SPECT patients, because exercise is the preferred modality for SPECT (leading to exclude more patients), while vasodilator stressor in our institution is preferred over exercise when indicating SE for suspect CAD, for the higher technical quality of images.

Finally data on the coronary flow velocity reserve (CFVR) on LAD during stress echocardiography are not available for this study. In our opinion the latter could have increased the sensitivity and specificity of cSE compared to SPECT.

6.2 Costs of the strategy. Considering the perspective of the Italian National Health System, the actual reimbursement established for diagnosis related group (DRG) concerning cSE, SPECT and coronary angiograms the total cost of the strategy is based on the cost of the functional tests and those related to coronary angiogram performed in case of positive test results for the confirmation of the diagnosis. Costs of the strategy were higher for SPECT because of greater costs associated with unnecessary coronary angiograms.

7. CONCLUSIONS

Our study demonstrates that SPECT and cSE have comparable significant prognostic capability for mortality and MI, with cSE demonstrating better diagnostic positive predictive value for CAD.

7.1 Clinical implications

Since SPECT apparently does not confer advantages in this specific setting, environmental protection considerations, financial and biological costs would apparently orient towards the use of cSE in subjects referred for pharmacologic stress imaging without a prior history of CAD.

Furthermore Stress echocardiography has several advantages, including the possibility of obtaining information on regional function and CFVR in the same sitting, with low cost and radiation-free, and the possibility to assess variety of applications and indications beyond coronary artery disease (28).

8. COMPETENCY IN MEDICAL KNOWLEDGE: In the current retrospective study, the long-term outcome of subjects with symptoms suggestive of CAD but no prior history of cardiac or coronary disease, who underwent either SPECT or contrast stress-echocardiography using vasodilator stressor was analyzed after careful matching of baseline characteristics. Since risk-stratification was similarly effective for both tests, and positive predictive value for CAD diagnosis was higher for contrast stress-echocardiography, we conclude and suggest that this cheaper test, with less impact on the environment and bio-hazard on the subject should be preferred in those subjects. Current results on subjects with no prior history of coronary or cardiac disease at this time should not be extrapolated to patients with prior MI, who were excluded from this study.

9. TRANSLATIONAL OUTLOOK: Further studies are needed to better appreciate how the tests that we have used for decades compare to each other regarding diagnostic and prognostic accuracy, but also according to their relative biological and environmental cost.

10. REFERENCES

1) Einstein AJ. Multiple opportunities to reduce radiation dose from myocardial perfusion imaging. Eur J Nucl Med Mol Imaging 2013;40: 649–651.

2) Picano E, Vano E, Rehani MM, Cuocolo A, Mont L, Bodi V et al. The appropriate and justified use of medical radiation in cardiovascular imaging: a position document of the ESC Associations of Cardiovascular Imaging, Percutaneous Cardiovascular Interventions and Electrophysiology. Eur. Heart J. 2014;35: 665-72

3)Thom H, West NE, Hughes V, et al; CECaT study group. Cost-effectiveness of initial stress cardiovascular MR, stress SPECT or stress echocardiography as a gate-keeper test, compared with upfront invasive coronary angiography in the investigation and management of patients with stable chest pain: mid-term outcomes from the CECaT randomised controlled trial. BMJ Open 2014;4: e003419.

4) Wolk MJ, Bailey SR, Doherty JU, et al; American College of Cardiology Foundation Appropriate Use Criteria Task Force. ACCF/AHA/ASE/ASNC/HFSA/HRS/SCAI/SCCT/SCMR/STS 2013 Multimodality Appropriate Use Criteria for the Detection and Risk Assessment of Stable Ischemic Heart Disease: A Report of the American College of Cardiology Foundation Appropriate Use Criteria Task Force. American Heart Association. American Society of Echocardiography. American Society of Nuclear Cardiology. Heart Failure Society of America. Heart Rhythm Society. Society for Cardiovascular Angiography and Interventions. Society of Cardiovascular Computed Tomography. Society for Cardiovascular Magnetic Resonance. and Society of Thoracic Surgeons. J Am Coll Cardiol 2014;63: 380-406.

5) Ladapo JA, Blecker S, Elashoff MR, et al. Clinical implications of referral bias in the diagnostic performance of exercise testing for coronary artery disease. J Am Heart Assoc 2013;2: e000505.

6) Neglia D, Rovai D, Caselli C, et al; EVINCI Study Investigators. Detection of significant coronary artery disease by noninvasive anatomical and functional imaging. Circ Cardiovasc Imaging 2015;8. pii: e002179. doi: 10.1161/CIRCIMAGING.114.002179.

7) Plana JC, Mikati IA, Dokainish H et al. A randomized cross-over study for evaluation of the effect of image optimization with contrast on the diagnostic accuracy of dobutamine echocardiography in coronary artery disease The OPTIMIZE Trial. JACC Cardiovasc Imaging 2008;1: 145-52.

8) Porter TR, Smith LM, Wu J, et al. Patient outcome following 2 different stress imaging approaches: a prospective randomized comparison. J Am Coll Cardiol 2013;61: 2446-55.

9) Health Quality Ontario. Stress echocardiography with contrast for the diagnosis of coronary artery disease: an evidence-based analysis. Ont Health Technol Assess Ser 2010;10: 1-59.

10) Argulian E, Po JRF, Uretsky S, et al. Comparison of the current reasons for undergoing pharmacologic stress during echocardiographic and radionuclide stress testing. J Nucl Cardiol 2017;24: 546-554.

11) Dawson D. Kaul S. Peters D. et al. Prognostic Value of Dipyridamole Stress Myocardial Contrast Echocardiography: Comparison with Single Photon Emission Computed Tomography. J Am Soc Echocardiogr 2009;22: 954–960.

12) Genders TS, Steyerberg EW, Hunink MG, et al. Prediction model to estimate presence of coronary artery disease: retrospective pooled analysis of existing cohorts. BMJ. 2012;344: e3485.

13) Gaibazzi N, Rigo F, Lorenzoni V, et al. Comparative prediction of cardiac events by wall motion. wall motion plus coronary flow reserve. or myocardial perfusion analysis: a multicenter study of contrast stress echocardiography. JACC Cardiovasc Imaging 2013;6: 1-12.

14) Gaibazzi N, Reverberi C, Lorenzoni V, et al. Prognostic value of high-dose dipyridamole stress myocardial contrast perfusion echocardiography. Circulation 2012;126: 1217-24.

15) Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging 2015;16:233-70.

16) Holly TA, Abbott BG, Al-Mallah M, et al; American Society of Nuclear Cardiology. Single photon-emission computed tomography. J Nucl Cardiol 2010;17: 941-73.

17) Germano G, Kavanagh PB, Waechter P et al. A new algorithm for the quantitation of myocardial perfusion SPECT. I: technical principles and reproducibility. J Nucl Med 2000;41: 712–9.

18) Hachamovitch R, Berman DS, Shaw LJ. et al. Incremental prognostic value of myocardial perfusion single photon emission computed tomography for the prediction of cardiac death: differential stratification for risk of cardiac death and myocardial infarction. Circulation 1998;97: 535-543.

19) Lauer MS, Blackstone EH, Young JB. Topol EJ. Cause of death in clinical research: time for a reassessment? J Am Coll Cardiol 1999;34: 618–620.

20) Ho D, Imai K, King G, Stuart E. Matching as Nonparametric Preprocessing for Reducing Model Dependence in Parametric Causal Inference. Political Analysis 2007;15: 199-236.

21) Marwick TH, Buonocore J. Environmental impact of cardiac imaging tests for the diagnosis of coronary artery disease. Heart. 2011;97: 1128–31. doi: 10.1136/hrt.2011.227884.

22) Braga L, Vinci B, Leo CG, Picano E. The true cost of cardiovascular imaging: focusing on downstream, indirect, and environmental costs. Cardiovasc Ultrasound. 2013;11: 10.

23) Wang EY, Dixson J, Schiller NB, Whooley MA. Causes and predictors of death inpatients with coronary heart disease (from the Heart and Soul Study). Am J Cardiol. 2017;119:27–34.

24) Hachamovitch R, Hayes SW, Friedman JD, Cohen I, Berman DS. Comparison of the short-term survival benefit associated with revascularization compared with medical therapy in patients with no prior coronary artery disease undergoing stress myocardial perfusion single photon emission computed tomography. Circulation. 2003;107: 2900-7.

25) Hendel RC, Friedrich MG, Schulz-Menger J, et al. CMR First-Pass Perfusion for Suspected Inducible Myocardial Ischemia. JACC Cardiovasc Imaging. 2016;9:1338-1348. doi: 10.1016/j.jcmg.2016.09.010.

26) Shaw LJ, Heller GV, Casperson P, et al. Gated myocardial perfusion single photon emission computed tomography in the clinical outcomes utilizing revascularization and aggressive drug evaluation (COURAGE) trial. Veterans Administration Cooperative study no. 424. J Nucl Cardiol. 2006;13: 685-98.

27) Gaibazzi N, Porter T, Lorenzoni V, et al. Effect of Coronary Revascularization on the Prognostic Value of Stress Myocardial Contrast Wall Motion and Perfusion Imaging. J Am Heart Assoc. 2017 May 31;6(6). pii: e006202. doi: 10.1161/JAHA. 117.006202.

28) Picano E, Ciampi Q, Citro R, D'Andrea A, Scali MC, Cortigiali L, et al. Stress echo 2020: the international stress echo study in ischemic and non-ischemic heart disease. Cardiovasc. Ultrasound 2017; 18:15

11. Figure Legends

Figure 1 shows Kaplan-Meier event-free survival curves for the outcome of myocardial infarction or death either in the overall group of patients who underwent either SPECT or cSE (left) or depending on SPECT and cSE results (right), either using crude data (upper) or matched populations (lower). The definition applied here for a positive SPECT for ischemia is any degree of ischemia, based on a summed difference score >1 (any degree of ischemia, mild included).

Figure 2 shows Kaplan-Meier event-free survival curves for the outcome of myocardial infarction or death depending on SPECT and cSE results, on the upper analysing crude data and on the lower after matching the two populations. In this case an ischemic SPECT was defined based on a summed difference score >4 (more than mild ischemia).

Figure 3 shows event-rates at increasing time (years) from the index-test after matching the two contrast stress-echocardiography (cSE) and SPECT groups based on demographics and clinical variables, as previously described. The outcome of myocardial infarction or death is significantly stratified by both cSE and SPECT. The definition applied here for an ischemic SPECT is again a summed difference score >4 (more than mild ischemia).

12. TABLES

Table 1. Baseline clinical characteristics and echocardiographic findings.

	cSE (n=890)	SPECT (n=497)	Total (n=1.387)	p-value
Age	65.1(11.5)	68.9 (11.1)	66.5 (11.5)	**<0.001**
Male gender	462(51.9%)	237(47.7%)	699(50.4%)	0.131
Probability of CAD, % (95% CI)	21 (11-36)	22 (12-39)	21 (11-37)	0.163
Smoke	228(25.6%)	72(14.5%)	300(21.6%)	**<0.001**
Hypercholesterolemia	472(53.0%)	229(46.1%)	701(50.5%)	**0.013**
Diabetes	211(23.7%)	124(25.0%)	335(24.2%)	0.604
Hypertension	637(71.6%)	383(77.1%)	1020(73.5%)	**0.026**
Aspirin	458(51.5%)	220(44.2%)	678(48.9%)	**0.01**
Plavix/Ticlop	61(6.9%)	29(5.8%)	90(6.5%)	0.46
Beta blockers	449(50.5%)	253(50.9%)	702(50.6%)	0.871
ACE/ARB	491(55.2%)	252(50.7%)	743(53.6%)	0.11
Statins	402(45.2%)	138(27.8%)	540(38.9%)	**<0.001**
LVEF <50%	80(9.0%)	59(11.9%)	139(10.0%)	0.084
Positive test (any degree of ischemia)	148 (16.6%)	102 (20.5%)	250 (18%)	0.070
Positive SPECT more than mild ischemia	-	68 (13.6%)	-	-

Data presented are mean value ±SD or number (%) of patients. CAD indicates coronary artery disease; LVEF. left ventricle ejection fraction; WM. wall motion; MP. myocardial perfusion; ACE-I. angiotensin converting enzyme inhibitors; ARBs. angiotensin receptor blockers. * Blood pressure≥140/90 mm Hg or treatment of hypertension. † Total cholesterol >200 mg/dl or treatment of hypercholesterolemia.

Table 2. Baseline clinical characteristics and echocardiographic findings after matching the 2 populations based on demographic and clinical variables. As expected, the baseline differences are not anymore statistically significant after the matching process.

	cSE (n=497)	SPECT	Total (n=994)	p-
Age	68.5 (10.5)	68.9 (11.1)	66.5 (11.5)	0,517
Male gender	237 (47.7%)	237 (47.7%)	699 (50.4%)	1
Smoke	79 (15.9%)	72 (14.5%)	300 (21.6%)	0,536
Hypercholesterolemi	227 (45.7%)	229 (46.1%)	701 (50.5%)	0,899
Diabetes	121 (24.4%)	124 (25.0%)	335 (24.2%)	0,825
Hypertension	368 (74.0%)	383 (77.1%)	1020 (73.5%)	0,268
Aspirin	222 (44.7%)	220 (44.2%)	678 (48.9%)	0,898
Plavix/Ticlop	29 (5.8%)	29 (5.8%)	90 (6.5%)	1
Beta blockers	247(49.7%)	253 (50.9%)	702 (50.6%)	0.703
ACE/ARB	239 (48.1%)	252 (50.7%)	743 (53.6%)	0.41
Statins	132 (26.6%)	138 (27.8%)	540 (38.9%)	0.669
LVEF <50%	60 (12.1%)	59 (11.9%)	139 (10.0%)	0.922
Positive test (any	69 (13.8%)	102 (20.5%)	171 (12.3%)	**0.005**
Positive SPECT	-	68 (13.6%)	-	-

Abbreviations as in Table 1.

Table 3. Univariate analysis of main clinical, medications and imaging parameters as predictors of cardiac ischemic events in the entire group of 1387 subjects who underwent either SPECT or cSE.

Univariate analysis	HR (95%CI)	95% CI		p-value
Clinical				
Age	2,108	1.459	3.047	**0.00**
Male Gender	1.057	1.039	1.076	**0.00**
Pre-test probability of CAD	1.032	1,024	1,040	**0.00**
Smoke	0.919	0.598	1.413	0.70
Hypercolesterolemia	0.890	0.630	1.257	0.51
Diabetes	1.730	1.204	2.485	**0.00**
Hypertension	1.891	1.185	3.017	**0.01**
Drugs				
Aspirin	1.880	1.313	2.690	**0.00**
Clopidogrel	2.316	1.372	3.911	**0.00**
Beta-Blockers	1.375	0.969	1.951	0.08
ACE-I/ARBs	1.427	1.000	2.036	0.05
Statin	1.223	0.863	1.732	0.26
Imaging				
Rest LVEF <50%	2.066	1.415	3.017	**0.00**
Overall cSE (vs overall SPECT)	0.892	0.626	1.270	0.53
Pos cSE vs Neg cSE	3.832	2.434	6.032	**0.00**
Neg SPECT vs Neg cSE	1.400	0.915	2.142	0.12
Pos SPECT vs Neg cSE	2.785	1.476	5.253	**0.00**

ACE-I/ARBs=ACE-inhibitors or angiotensin blockers; LVEF=left ventricle ejection fraction. cSE= contrast stress-echocardiography SPECT is defined as ischemic only for more than mild ischemia (SDS>4).

Table 4. Univariate and multivariable analysis of main clinical, medications and imaging parameters as predictors of cardiac ischemic events in the group of 994 subjects obtained after matching the 2 groups (497 SPECT plus 497 matched cSE) who underwent SPECT or cSE.

Univariate analysis	HR	Inf CI95%	Sup CI95%	p-value
Clinical				
Age	1.059	1.036	1.082	**0.00**
Male Gender	2.112	1.392	3.205	**0.000**
Smoke	1.200	0.711	2.026	0.494
Pre-test probability of CAD	1.032	1,022	1,041	**0.00**
Hypercolesterolemia	0.814	0.543	1.219	0.318
Diabetes	1.788	1.181	2.706	**0.006**
Hypertension	1.814	1.046	3.145	**0.034**
Drugs				
Aspirin	1.684	1.127	2.516	**0.011**
Clopidogrel	1.835	0.924	3.643	0.083
Beta-Blockers	1.434	0.957	2.149	0.080
ACE-I/ARBs	1.417	0.947	2.120	0.090
Statin	1.290	0.842	1.977	0.242
Imaging				
Rest LVEF reduction (<50%)	2.304	1.432	3.707	**0.001**
Overall cSE (vs overall SPECT)	0.947	0.635	1.412	0.791
Pos cSE vs Neg cSE (reference)	3.074	1.640	5.761	**0.000**
Neg SPECT vs Neg cSE (reference)	1.172	0.734	1.871	0.506
Pos SPECT vs Neg cSE (reference)	2.343	1.206	4.553	**0.012**

Multivariable analysis	HR (95%CI)	Inf CI95%	Sup CI95%	p-value
Age	1.060	1.036	1.085	**0.000**
Male Gender	2.227	1.459	3.400	**0.000**
Smoke	-			
Pre-test probability of CAD				
Hypercolesterolemia	-			
Diabetes	1.533	1.006	2.335	**0.047**
Hypertension	1.486	0.846	2.609	0.168
Aspirin	-			
Clopidogrel	1.193	0.790	1.800	0.401
Beta-Blockers	-			
ACE-I/ARBs	-			
Statin	-			
Rest LVEF reduction (<50%)	-			
Overall cSE (vs overall SPECT)	1.816	1.121	2.944	**0.015**
Pos cSE vs Neg cSE (reference)	-			
Neg SPECT vs Neg cSE (reference)	1.982	1.047	3.750	**0.036**
Pos SPECT vs Neg cSE (reference)	1.068	0.667	1.710	0.784
	1.950	0.998	3.810	**0.051**

ACE-I/ARBs=ACE-inhibitors or angiotensin blockers; LVEF=left ventricle ejection fraction. cSE= contrast stress-echocardiography SPECT is defined as ischemic only for more than mild ischemia (SDS>4).

Table 5. Multivariable cox models to predict outcome in SPECT and cSE groups after matching the cSE population based on the SPECT population.

SPECT group n=497	MULTIVARIATE			
	HR	**CI 95%**		**p-value**
Age	1.056	1.024	1.089	**0.001**
Gender	3.231	1.738	6.005	**0.000**
Hypertension	2.301	0.968	5.466	0.059
LVEF<50%	1.844	0.941	3.612	0.074
Positive (SDS>4) vs Neg	1.974	1.019	3.824	**0.044**

ECHO matched group N=497	MULTIVARIATE			
	HR	**CI 95%**		**p-value**
Age	1.064258	1.027	1.102	**0.001**
Diabetes	1.557312	0.852	2.844	0.15
LVEF<50%	1.860616	0.937	3.692	0.076
Positive vs Neg cSE	2.314893	1.224	4.375	**0.01**

LVEF=left ventricle ejection fraction. cSE= contrast stress-echocardiography. SPECT is defined as ischemic only for more than mild ischemia (SDS>4).

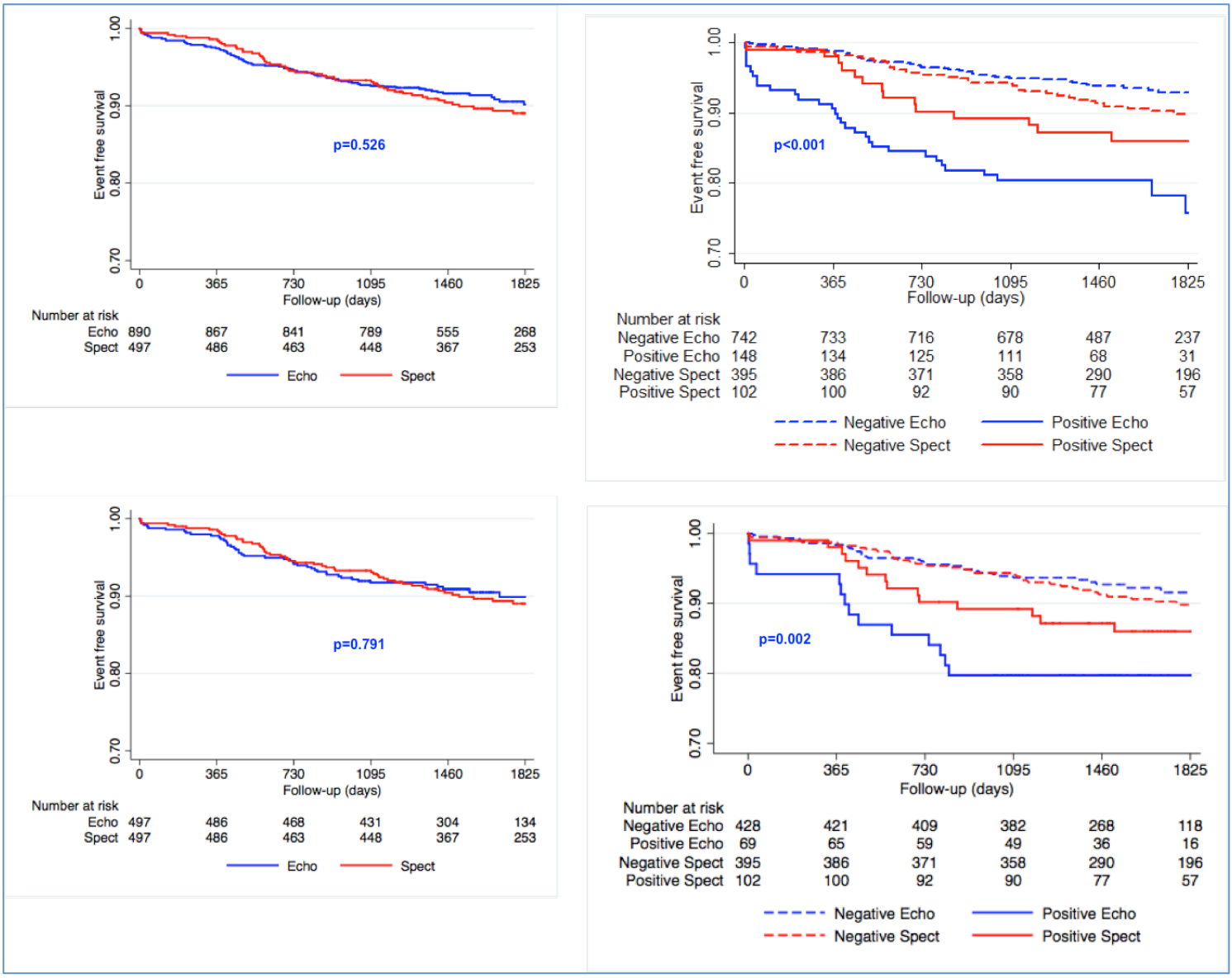
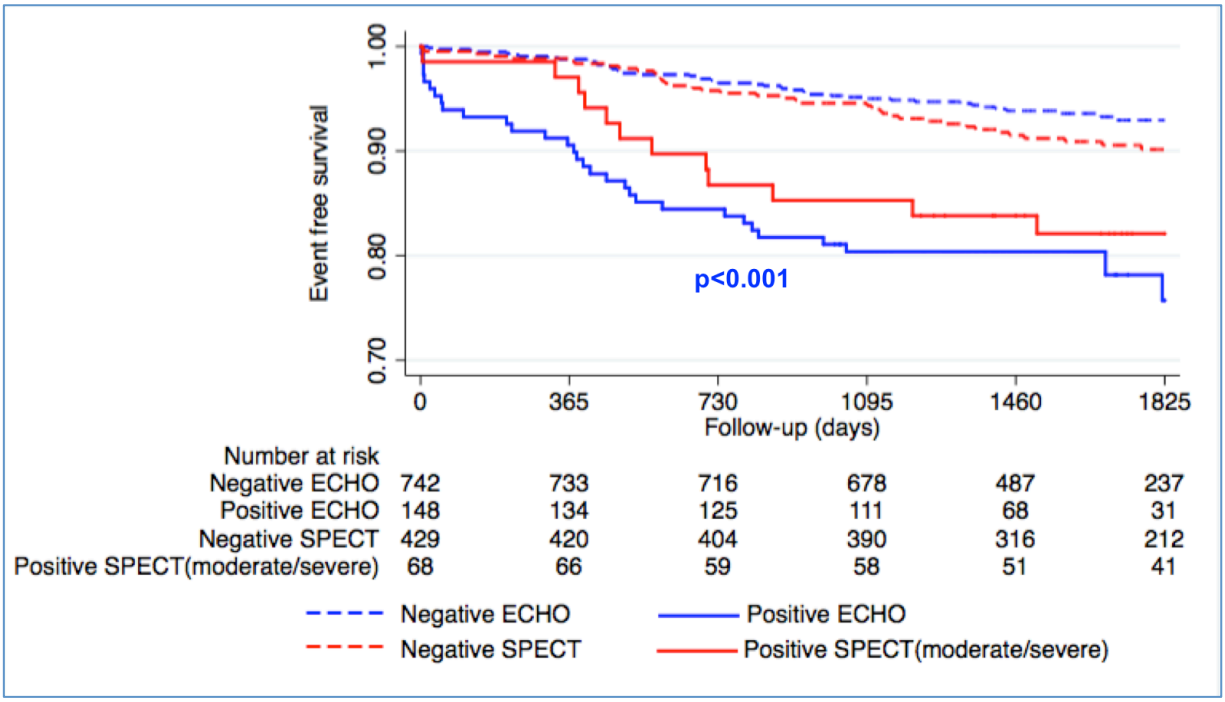

Event free survival
1.00
0.90
0.80
0.70
p=0.526
0
365
730
1095
1460
1825
Follow-up (days)
Number at risk
Echo 890 867 841 789 555 268
Spect 497 486 463 448 367 253
Echo
Spect
p<0.001
Number at risk
Negative Echo 742 733 716 678 487 237
Positive Echo 148 134 125 111 68 31
Negative Spect 395 386 371 358 290 196
Positive Spect 102 100 92 90 77 57
Negative Echo
Positive Echo
Negative Spect
Positive Spect
p=0.791
Number at risk
Echo 497 486 468 431 304 134
Spect 497 486 463 448 367 253
p=0.002
Number at risk
Negative Echo 428 421 409 382 268 118
Positive Echo 69 65 59 49 36 16
Negative Spect 395 386 371 358 290 196
Positive Spect 102 100 92 90 77 57

Figure 1.


Event free survival
1.00
0.90
0.80
0.70
p<0.001
0
365
730
1095
1460
1825
Follow-up (days)
Number at risk
Negative ECHO 742 733 716 678 487 237
Positive ECHO 148 134 125 111 68 31
Negative SPECT 429 420 404 390 316 212
Positive SPECT(moderate/severe) 68 66 59 58 51 41
Negative ECHO
Positive ECHO
Negative SPECT
Positive SPECT(moderate/severe)

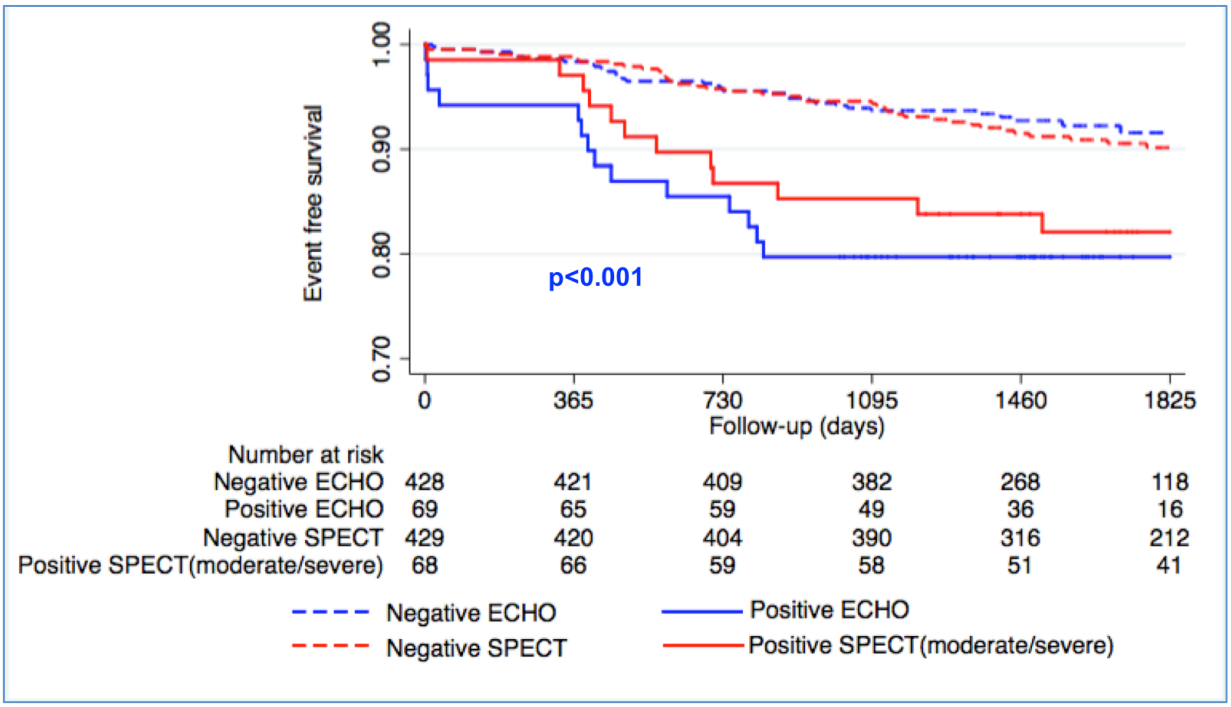
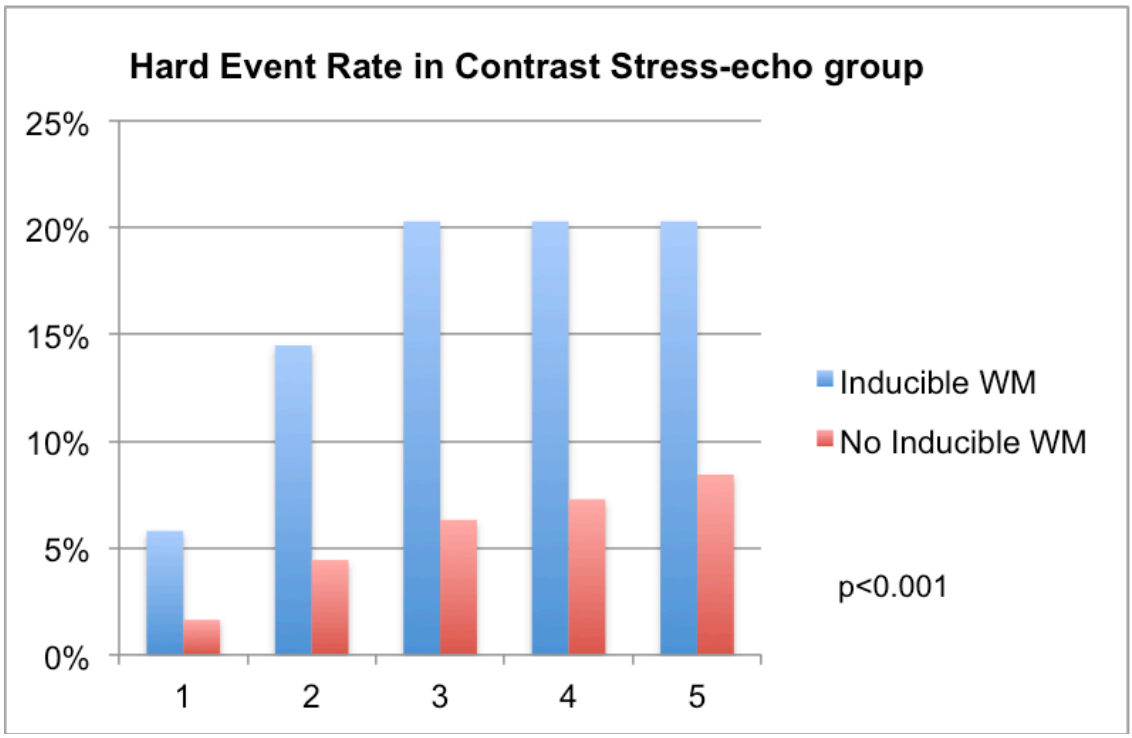

Event free survival
1.00
0.90
0.80
0.70
p<0.001
0
365
730
1095
1460
1825
Follow-up (days)
Number at risk
Negative ECHO 428 421 409 382 268 118
Positive ECHO 69 65 59 49 36 16
Negative SPECT 429 420 404 390 316 212
Positive SPECT(moderate/severe) 68 66 59 58 51 41
Negative ECHO
Positive ECHO
Negative SPECT
Positive SPECT(moderate/severe)

Figure 2.


Hard Event Rate in Contrast Stress-echo group
25%
20%
15%
10%
5%
0%
1
2
3
4
5
Inducible WM
No Inducible WM
p<0.001

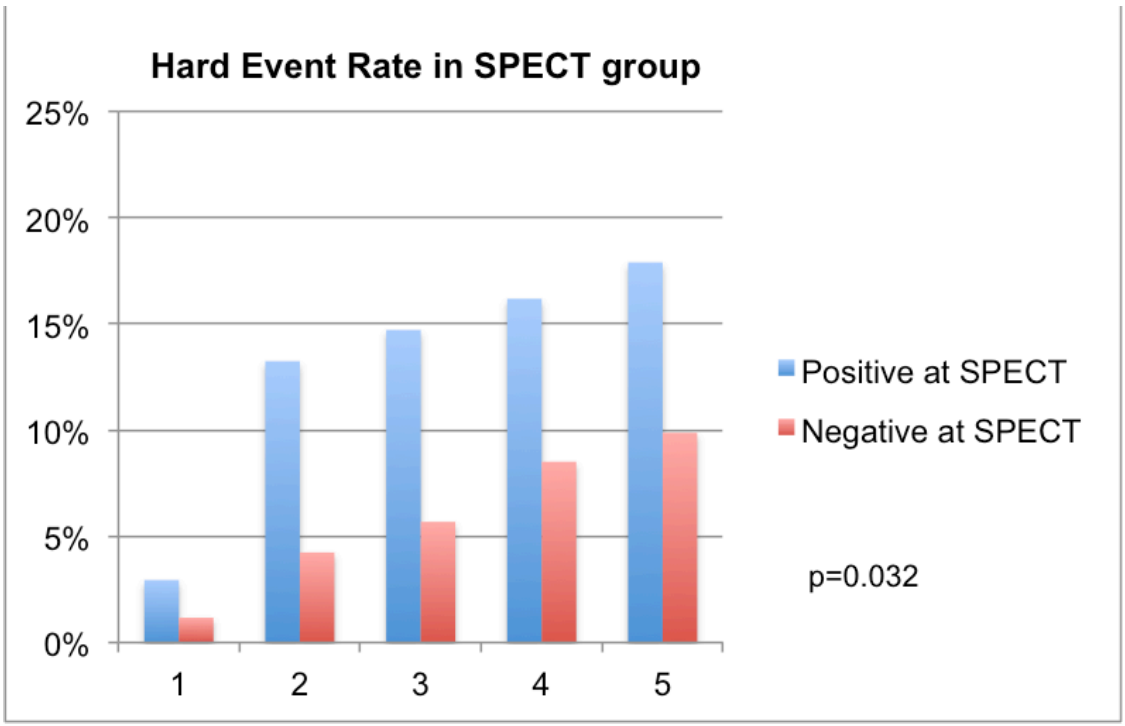

Hard Event Rate in SPECT group
25%
20%
15%
10%
5%
0%
1
2
3
4
5
Positive at SPECT
Negative at SPECT
p=0.032

Figure 3

15. RINGRAZIAMENTI

Il primo ringraziamento va all'amico Nicola Gaibazzi.

A lui devo il sostegno scientifico in questo percorso di formazione accademica, l'acquisizione di una mentalità scientifica e la consapevolezza dei limiti e degli obiettivi da perseguire.

Una persona "folle" che sposa il mio modo di sentire le cose. Vittima di un "sano bipolarismo" appartenente solo a chi mette il cuore nelle cose.

Stay hungry stay foolish (Steve Jobs).

Ringrazio il Prof. Riccardo Volpi e il Prof. Paolo Sansoni perchè attraverso i loro insegnamenti ho superato tanti miei limiti. Mi hanno accompagnato, sostenuto, dato fiducia non senza richiami, in questo dottorato e nella specializzazione.

Ringrazio I miei genitori che mi hanno reso ricercatore molto prima di adesso, lasciandomi libero di seguire la mia strada.

Ringrazio mia moglie che mi è sempre stata vicino, per la forza e per il sostegno che mi ha sempre dato e che continua a darmi nei momenti belli ed in quelli difficili, non senza sacrificio.

Ringrazio mio fratello Andrea perchè mi ha insegnato ad essere forte.

Ringrazio tutti Coloro che mi amano, poichè *per* queste persone e *con* queste persone porto avanti i sogni, le paure e le soddisfazioni della vita. Nessuna maratona si corre da soli.

Ringrazio infine il mio cuore perchè è il motore del mio essere e mi permette di vivere secondo ciò che mi fa sentire me stesso.

Da oggi sono Ricercatore, PhD.

Lo sono oggi e lo sarò per sempre,

ma forse lo sono sempre stato,

perchè io credo la ricerca come una attività libera,

che non ha bisogno di titoli, ma solo di cuore.

Carmine