Title: A p**R**ospectiv**E**, ob**S**ervati**O**nal, mu**L**ticenter st**U**dy to assess the effects of differen**T** ant**I**- thromb**O**tic regimens in subjects with left ve**N**tricular thrombus (**RESOLUTION** Registry). Insights From the Italian Society of Echocardiography and Cardiovascular Imaging Research Network

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Background and rationale

Left ventricular thrombus (LVT) development is a feared complication following different forms of heart disease, with potential for significant morbidity and mortality. It remains strongly associated with myocardial infarction[1] and might increase the risk of thrombo-embolic events, including stroke[2]. Patients are also considered to be at high risk for LVT formation if they have a reduced LV ejection fraction (< 40%)[3] due to cardiomyopathy or infiltrative myocardial disease or antero-apical akinesis/dyskinesis[4].

Thrombus resolution requires appropriate anticoagulation treatment, and current guidelines recommend vitamin K antagonists (VKAs) as the first-choice therapy in these patients population[5]. Nevertheless, there are no contemporary registry evaluating the safety and efficacy of anticoagulation in the treatment of LVT, and clinicians must rely on single-center, tertiary care experience and available epidemiologic-generated data to guide treatment. This gap in knowledge is notable considering that treatment options for LVT have become more complicated, both for the characteristics of patients, progressively older with multiple comorbidities, who often require concomitant chronic anticoagulation and various antiplatelet therapy schemes, and for the emergence of the direct oral anticoagulants (DOACs) in the setting of thromboembolic prophylaxis for atrial fibrillation[6] or pulmonary embolism[7]. However, the efficacy data of DOACs in the treatment of LVT are limited to small case series and one meta-analysis of case reports[8]. Also, randomized clinical trials have confirmed that triple therapy increases bleeding rates compared with less potent antithrombotic regimens after myocardial infarction, and observational data suggest that

triple therapy regimens may not prevent LVT formation. Therefore, in the contemporary era, optimal antithrombotic strategies in LVT may require reconsideration.

Left ventricular thrombosis resolution and reduction

If an LVT is detected, anticoagulation is essential to prevent systemic thromboembolism. Among those with follow-up imaging within 1 year, LVT resolution occurred in ≈75% who received VKAs[9] or DOACs[8]. However, ***data about LVT resolution vs reduction, the association of LVT resolution with embolic event and the embolic avoidance without LVT resolution are limited or lacking.***

One of the reasons for this scarcity of data lies in the limits of standard echocardiography in the assessment of LVT. Indeed, sensitivity of routine transthoracic echocardiography has been reported in the range 21–94.7%, while specificity was 94–98.3%. The use of ultrasound contrast agents greatly improves the accuracy from 82% to 92% when compared to cardiac magnetic resonance[10]. This is especially true in patients with a layered thrombus (thrombus with no protruding element and which conforms to area of the ventricular wall). A protruding and mobile thrombus indicates a higher embolic risk compared with a flat, immobile thrombus. ***Therefore, ultrasound contrast should be used in all patients with suboptimal ultrasound windows for detection and follow-up of LVT[11].***

The objective of the study is to assess the effects of different anticoagulant regimens, including unfractioned heparin, low-molecular weight heparin, VKAs and DOACs, on the resolution or reduction of LVT, as assessed by echocardiography (with ultrasound contrast in patients with suboptimal ultrasound windows) and correlations with outcomes. The use of different anticoagulant regimens and ultrasound contrast will be left at the discretion of attending physicians, according to good clinical practice.

It is important however to point out that with a lack of clear randomized clinical trial data and great variability in the presentation and associated complications of LVT, individualized approaches will continue to be necessary. Current treatment strategies for LVT are also unclear and require examination.

Study design

Echocardiography analyses

To identify factors predicting incremental utility of tailored imaging for LVT, a contrast echo will be performed in all patients with suboptimal ultrasound windows when LVT are not clearly documented or excluded on non-contrast images and to demonstrate resolution or reduction of the LVT during follow-up. Contrast opacification particularly facilitates the identification of apical abnormalities. This is because native tissue harmonic echocardiography is unable to overcome the noise, clutter and reverberation artefacts in the near field as tissue harmonic signals are weak at the near field[13]. Over 10 years of use of contrast on millions of patients established the safety of contrast. In a large retrospective analysis of 18000 patients, of which one-third received contrast agent in the acute setting, there was no significant difference in mortality in patients who received contrast vs. those who did not[14]. Side effects have been noted with contrast agents, but they are usually mild and transient. Serious allergic reactions have been observed at a very low incidence (estimated to be 1:10 000). Allergic reactions have been reported within 30 min. Table lists risk categories observed during usage of competing investigations[15]. Adverse events are rare (seen in between 1 in 1000 and 1 in 10 000 patients) and usually mild (headache, nausea, dizziness, taste disturbances, paraesthesia, chest discomfort and reactions at the injection side). They are usually transient and do require any treatment apart from reassuring the patients.

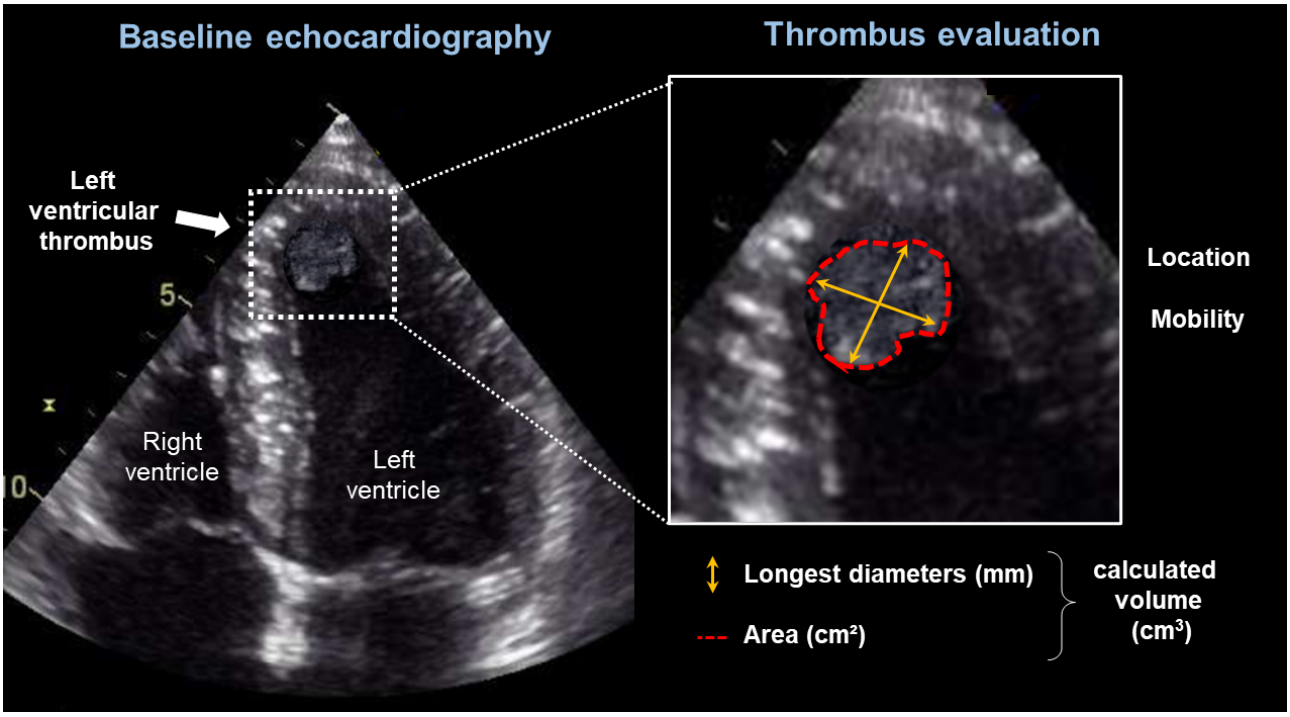
Table. Incidence of severe anaphylaxis by substance class as defined by the International Collaborative Study of Severe Anaphylaxis[15].

Risk Category	Incidence	Substance Class
Low	0.005% - 0.015%	Analgesics
		Antibiotics
		MRI-Contrast Media
		Echo contrast agents
Medium	0.03% - 0.1%	Penicillin IV
		Blood Dextrane
		Pentoxyphylline
		Iodine-Contrast Media
High	> 0.1%	Plasma
		Streptokinase

Thrombus will be diagnosed on using established criteria[16,17]. Particularly, an LVT will be detected based on its location within the LV cavity and appearance with no signal intensity (ie, homogeneously black) due to the absence of vascularity, surrounded by structures with contrast uptake (LV cavity and myocardium).

The size of the thrombus was measured as the largest 2-dimensional area available on the index echocardiogram. ***LVT diameters (mm), area (cm^2), and volume (cm^3)*** were assessed for each transthoracic echocardiogram considering the mean of 3 measures to study LVT evolution over time (Figure 2). Mobility and location of LVT were also defined. LVT will be categorized as ***protuberant***, if its borders are distinct from the adjacent endocardium and it protruded into the LV cavity, or as ***mural***, if its borders are contiguous with the adjacent endocardium, as described previously[18-20]. If >1 LVT are present, the morphology will be characterized as protuberant if any thrombus will protrude into the LV cavity. An LVT will be categorized as ***mobile*** if it is noted to be independently mobile. LVT ***area and volume*** will be quantified by planimetry (Figure 2). A calcified LVT was defined as a persistent left ventricular mural thrombus encapsulated by thickened and calcified endocardium.

Figure. Methodological aspects of left ventricular thrombus echocardiographic evaluation.



Echocardiography will be also scored for **diagnostic quality** using a previously established 9-point scale. The scale comprised separate scores for endocardial definition (1 = poor; 2 = fair; and 3 = excellent), cavity artifacts (1 = present and obscuring full LV assessment; 2 = present but interpretable; and 3 = absent), number of apical views (1 = single orientation and 2 = at least 2 orientations), and number of LV segments imaged (1 = all segments).

Regional wall motion will be scored using an American Heart Association/American College of Cardiology 17-segment model, for which segmental contraction are graded as follows: 0 = normal; 1 = mild hypokinesis; 2 = moderate hypo- kinesis; 3 = severe hypokinesis; 4 = akinesis; and 5 = dyskinesis. **Apical LV wall motion scores** will be calculated on noncontrast and contrast echo by summing segmental scores within the apical LV and true apex (total 5 segments)[21].

Timing of follow-up: an ultrasound contrast echocardiogram will be performed, according to good clinical practice, at day 14+/- 4 (approximately 2 weeks), 42+/- 7(approximately 6 weeks) and 91+/- 14 (approximately 13 weeks) from the start of the anticoagulant treatment, to ascertain the thrombus resolution. These timing of noninvasive examination is based on evidence from literature.

Study objectives

Primary objectives

- Evaluate LVT ***regression*** under anticoagulation therapy and the time needed for total LVT regression using sequential contrast echocardiography
- The independent correlates associated with total LVT regression
- The impact of LVT regression on thromboembolism, bleeding, and mortality and factors associated with major cardiovascular adverse events (MACEs)

Secondary objectives

- Evaluate LVT ***persistence (increased dimension, stable dimension, partial regression)***
- The independent correlates associated with total LVT persistence
- The impact of LVT persistence subtypes on MACEs

Safety Objective

- Determine the safety of different anticoagulant regimens as measured by subject incidence of major or clinically relevant non-major bleeding events.

Endpoint definitions

Total LVT regression: complete disappearance of LVT on all contrast echocardiography views at the last available follow-up. LVT persistence: classified as an increased thrombus dimension, a stable thrombus, or a partial thrombus regression at the last available follow-up.

MACEs: composite of all-cause death, myocardial infarction, or acute peripheral artery emboli (any embolic complications defined as the composite of ischemic stroke or transient ischemic attack, acute coronary emboli, or acute peripheral artery emboli: limb, renal, or digestive arteries)

Bleeding will be defined in accordance with The International Society on Thrombosis and Haemostasis (ISTH)/Scientific and Standardization Committee (SSC) in non-surgical patients[22,23]:

Major bleeding

1. Fatal bleeding

and/or

2. Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome

and/or

3. Bleeding causing a fall in hemoglobin level of 2 g/dL or more, or leading to transfusion of two or more units of whole blood or red cells.

Clinically relevant non-major bleeding

Acute or subacute clinically overt bleed that does not meet the criteria for a major bleed but prompts a clinical response, in that it leads to at least one of the following:

- A hospital admission for bleeding, or
- A physician guided medical or surgical treatment for bleeding, or
- A change in antithrombotic therapy (including interruption or discontinuation of study drug).

Selection of Population and Patients Enrolment

No data will be collected before detailed information is given to the patient and written informed consent is signed. All consenting patients seen in the out- /in-patient clinic will be included.

Inclusion Criteria

Presence of LVT, as assessed on transthoracic echocardiography ± ultrasound echocontrast, regardless of etiology and LV ejection fraction.

Exclusion Criteria

- Subjects younger than 18 years
- Active bleeding or high risk for bleeding contraindicating treatment with anticoagulants
- Any clinically unstable cardiac condition prior to echocardiography
- Known allergy to 1 or more ingredients of contrast agent

Current or previous participation to cardiovascular or non-cardiovascular trial is not excluding the patient from participation in the RESOLUTION study.

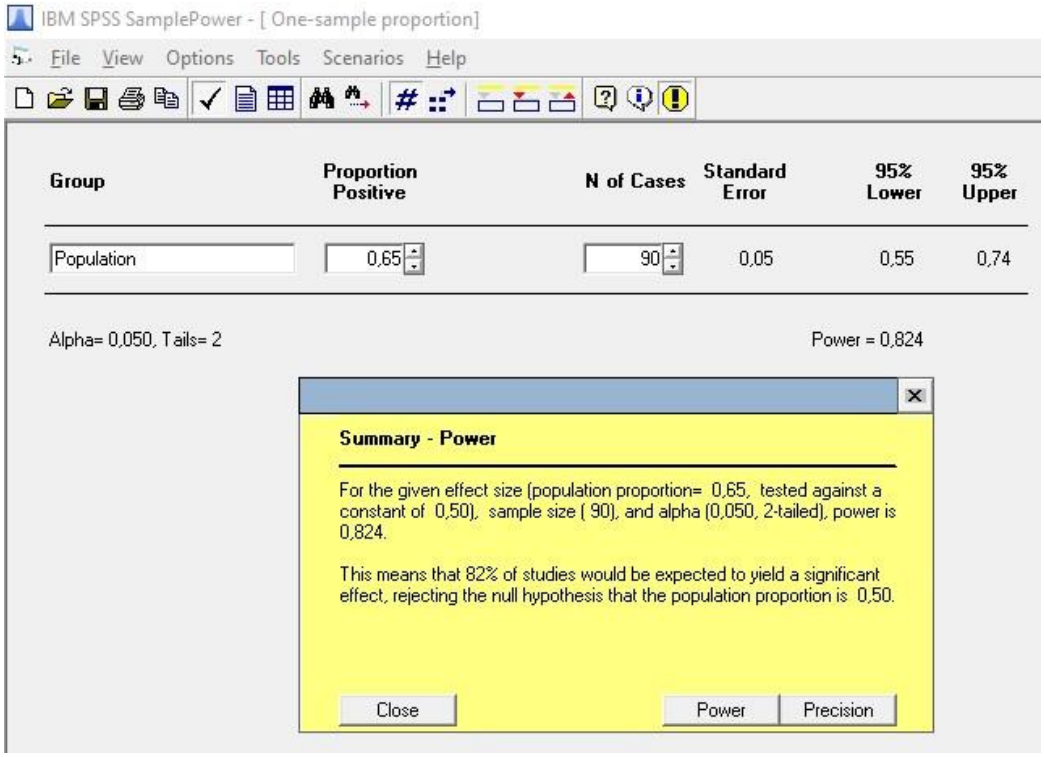
Centers involved and duration of enrollment period

Approximately 10-15 centers will be involved in this observational study. The expected duration of enrollment is 12 months.

Study Design and Sample size

The RESOLUTION study is a prospective, multicentre and observational registry of patients with LVT formation presenting to cardiology centres in Italy who will be interested in taking part in the study. It will involve cardiology Units that regularly follow and/or admit patients with a wide variety of cardiac pathology.
Ten to fifteen centres will be appointed and accepted on a voluntary basis. It is expected that 8-10 patients with LVT will be observed per center per year. Accordingly, 80-150 patients should be enrolled over a period of 12 months. This sample size should allow to generate hypothesis for improve our therapeutic approach to LVT: in particular the rate of LVT resolution at 3 months, expected in about 60-70 % of patients, should allow sufficient data in this population to evaluate the efficacy of therapy. On the other hand, the percentage of LVT persisting after 3 months, expected in 30-40 %, should allow to know the features responsible for this inadequate response.

The IBM-Sample PowerTM ver. 3.0 software will be used to calculate the sample size; sampling tests will be accepted at the power level $\beta >= 80\%$, $\alpha = 5\%$, and tests with two tails (see below).



Patients will be followed until one year after enrolment. There will be no attempt to interfere with the routine clinical care of the patient who, according to disease's condition, will be expected to attend at least one visit during the follow-up. A visit close to 12 months after the in- or outpatient entry visit will be recommended in order to collect information on morbidity and mortality. A phone call can replace the follow-up clinical visit in cases where the patient cannot attend centre for clinical or logistical reasons.

Decisions on drug prescriptions and indications to perform therapeutic procedures will be left to participating cardiologists, according to international guidelines and good clinical practice. No specific protocols or recommendations for treatment will be made during this observational study.

Ethical Issues

All centres will require ethics approval; the national coordinator will be responsible for obtaining the approval of the local and national review boards for this study, if necessary.

Patient Consent

All patients will be approached by the centre investigator and will be asked for their written informed consent to participate in the RESOLUTION study on LVT.

No data will be collected before written detailed information is given to the patient and a signed informed consent is obtained.

In centres where written informed consent is not mandatory for patient participation in a registry, written informed consent will not be required but this should be documented in the ethics application and approved by the ethics board, according to the local rules.

For those patients who will be admitted with severe clinical condition and not able to consent at time of admission, information and written consent will be obtained from a legally authorized representative if allowed by the ethics board. Patients will have to give consent as soon more favorable clinical conditions allow them to receive appropriately the study information.

Patient or legally acceptable representative will be given a copy of the signed informed consent.

Protection of Human Subjects

The RESOLUTION study is an observational study that does not dictate the manner in which patients are treated in order to achieve the LVT resolution. Physicians may decide to evaluate and manage outpatients and inpatients with LVT in the most appropriate way, according to the standard of care.

In case of refusal, the patient will not be enrolled in the study and their data will not be collected.

The main database will be secured according to current standards to ensure both ethical and integrity requirements of the data. Of note, the RESOLUTION does not require the transmission of identification data outside the participating centers. The data collected will be anonymous. Each patient will be assigned a unique identification number and no other identification variables will be entered. The identity of the patient will remain at the participating center as confidential information. Information aimed at identifying the individual patients of the study will not be collected or stored in the database. All confidential information will be password protected for electronic data or stored in secure places for paper data. For these reasons, a high level of security is assured. To maintain these high levels of security at the same time as data reliability, each researcher will have a single personal login and password to access patient information (REDCap Consortium). There will not be a collection of data outside the collection tools, which will take place through a web platform, absolutely secure based on current standards concerning the ethical requirements and data integrity.

Pharmacovigilance

In this observational study there is no intervention and no adverse event or serious adverse event will be expected.

Statistical Analyses

All the patients enrolled and with complete data will be included in the analyses. Since this is an observational study, descriptive summaries will be presented for all the patients and for subgroups of patients. Parametric and non-parametric statistical tests may be carried out for exploratory purposes, as appropriate. Multivariable analyses such as, but not limited to, linear, logistic and Cox regressions and generalized estimating equations, as well as prognostic calibration and discrimination statistics, may be used to explore the relationship between different baseline covariates and post-baseline endpoints, as appropriate. Missing baseline data will be handled by multiple imputations and in sensitivity analyses, with complete case analyses.

A Statistical Analysis Plan will be generated for the registry to set complete statistical considerations and analysis.

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