

Infrared Thermography and Flow Mediated Dilation Measure by Ultrasound as Predictors of Cardiovascular Risk

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Resumo – Termografia por radiação de infravermelhos (TRIV) associada com manobra de isquemia e reperfusão foi capaz de discriminar o risco futuro de acidente vascular cerebral e aterosclerose subclínica (placas carotídeas) em experimentos anteriores. No entanto, não se sabe se o mecanismo pelo qual esses resultados foram obtidos foi devido à disfunção endotelial. Estudamos a associação entre TRIV e um método bem estabelecido de análise de função endotelial (US braquial) e avaliamos a associação entre TRIV e o escore de risco global (Escore de Risco Global/Framingham). **Métodos:** 97 voluntários foram alocados aleatoriamente: 53% mulheres, 60 anos (24-85 anos), 60 hipertensos (62%), 10 fumantes (10%) e 21 diabéticos (22%); A TRIV foi analisada por 2 médicos que, como os outros participantes, eram cegos aos outros testes. A TRIV seguiu o protocolo: 1) obtenção dos termogramas das mãos em repouso 2) compressão suprassistólica a 200mmHg por 5 minutos do braço direito 3) gravação dos termogramas das mãos na isquemia máxima 4) liberação da braçadeira do voluntário 5) registro de termogramas a cada minuto nos próximos 5 minutos. Foram obtidas três variáveis numéricas (temperatura de rebote, taxa de isquemia máxima e taxa de hiperemia reativa) e 1 categórica (neuroreatividade, NR). US braquiais foram realizadas por um único médico experiente no método e seguiram o protocolo: 1) medição do calibre da artéria braquial em seção longitudinal a 2cm da fossa ulnar esquerda no pico da onda ECG R 2) compressão suprassistólica do antebraço esquerdo por 5 minutos 3) descompressão do antebraço 4) medição do calibre da artéria braquial durante o segundo e quinto minuto após a descompressão do antebraço. O US braquial permitiu a obtenção de uma variável numérica, mediada por fluxo dilatado (DFM). Os voluntários foram estratificados por 2 cardiologistas através do GRS. **Resultados:** O coeficiente de correlação de Spearman (r) foi uma associação positiva, significativa e moderada entre TRIV e DFM, sendo a temperatura de recuperação a variável de melhor desempenho, $r = 0.52$ ($p < 0.001$). Observamos uma maior prevalência de hiperatividade simpática (NR alterada) nos voluntários com menor DFM, bem como uma maior prevalência de voluntários com baixo risco de EGR e NR normal (teste exato de Fischer, $p < 0,001$). O ponto de corte para discriminação de indivíduos de alto risco (RGEs) foi de 1,25°C e 7,14% para TRIV e DFM, respectivamente. **Conclusões:** Encontramos uma correlação positiva, significativa e moderada entre os dois métodos. Além disso, nosso estudo demonstrou uma boa associação entre o Escore de Risco Global/Framingham com achados obtidos na TRIV.

Palavras-chave: endotélio vascular; morbidade; raios infravermelhos

Abstract – Infrared radiation thermography (TRIV) associated with ischemia and reperfusion maneuver was able to discriminate the future risk of stroke and subclinical atherosclerosis (carotid plaques) in previous trials. However, it is not known if the mechanism by which such results were obtained was due to endothelial dysfunction. We studied the association between TRIV and a well-established method of endothelial function analysis (brachial US) and additionally assessed the association of TRIV with the global risk score (Global risk Score/Framingham). **Methods:** 97 volunteers were randomly allocated: 53% women, age 60 (24-85 years), hypertensive 60 (62%), smoking 10 (10%) and diabetes 21 (22%). TRIV were analyzed by 2 physicians who, like the other participants, were blind to the other tests. TRIV followed the protocol: 1) obtaining thermograms of the hands at rest 2) supra-systolic compression at 200mmHg for 5 minutes of the right arm 3) recording of the thermograms of the hands in maximal ischemia 4) release of the arm cuff of the volunteer 5) Record minute hand thermograms in the next 5 minutes. Three numerical variables were obtained (rebound temperature, maximal ischemia rate and rate of reactive hyperemia) and 1 categorical (neuro reactivity, NR). Brachial US was performed by a single physician experienced in the

method and followed the protocol: 1) measurement of the caliber of the brachial artery in longitudinal section 2 cm from the left ulnar fossa at the peak of the ECG R wave 2) supra-systolic compression of the forearm Left for 5 minutes. 3) Forearm decompression 4) Measurement of the caliber of the brachial artery during the second and fifth minutes after the decompression of the forearm. Brachial US allowed the achievement of a numerical variable, dilated flow mediated (DFM). Volunteers were stratified by 2 cardiologists through the GRS. **Results:** Spearman's correlation coefficient (r) was a positive, significant and moderate association between TRIV and DFM, with rebound temperature being the best performing variable, $r = 0.52$ ($p < 0.001$). We observed a higher prevalence of sympathetic hyperactivity (altered NR) in the volunteers with lower DFM, as well as a higher prevalence of volunteers with low risk for EGR and normal NR (Fischer's exact test, $p < 0.001$). The cutoff point for discriminating high risk individuals (RGEs) was 1.25°C and 7.14% for TRIV and DFM, respectively. **Conclusions:** We found a positive, significant and moderate correlation between the two methods. In addition, our study demonstrated a good association between the global risk score / Framingham with thermographic findings obtained in the TRIV.

Keywords: endothelial vascular; morbidity; infrared rays

INTRODUCTION

Recently, two studies published using digital infrared thermal imaging (TRIV) associated with provocative maneuver ischemia and reperfusion arm demonstrated exist association between changes of the nervous system behavior autonomic sympathetic and microvascular reactivity with the risk of cardiovascular disease.^{1,2}

In both studies, the presence of sympathetic overactivity (SO) observed by the fall in the average temperature in the distal phalanges (ROI) of the left hand during systolic above compression of the right arm between the moments 0 (baseline) until the fifth minute (maximum ischemia) was the variable that had the best ability to predict the risk for future cardiovascular events. The existence of SO gave a bent of a future stroke risk and a chance five and a half times to present of atheroma plaques in the carotid arteries in a population of outpatients.

Other TRIV variables such as temperature rebound, ischemia maximum rate and reactive hyperemia rate also showed a good association with the risk of stroke as well as the thickening of the carotid arteries. It has been shown in these two trials no difference according to gender to evaluate the results obtained in TRIV.³

These findings suggest there is a relationship of thermal variables obtained on the surface of the skin (microcirculation) with the behavior of endothelial cells (for instance: The rebound temperature to the degree of reactive hyperemia and so with the level of nitric oxide released by the endothelium) however mechanistic studies are required for confirmation is given that maintains the microcirculation different characteristics from those found in the large conductance vessels.

Dilation flow-mediated brachial artery (FMD) is a method well established in studies to assess endothelial function since it was the first non-invasive method used for this purpose, it has shown to be

strongly influenced when used inhibitors and agonists NO (L-NMMA and e-NOS) and there is already an international policy of standardization to carry out this method.^{4,5,6,7}

This essay aims to study the association between these two methods in order to understand the behavior of macrocirculation (FMD) and microcirculation (TRIV) after reactivity maneuvers can be associated thus suggesting the relationship of thermographic measurements with endothelial function and also check the correlation of these two tests with cardiovascular global risk score (Framingham), classification recommended by the Brazilian Society of Cardiology (SBC)⁸ and other international medical societies

METHODOLOGY

Materials and methods

A cross-sectional study, allocating patients without anatomical or sensory changes of hands, with the inclusion of patients with temperature of hands above 19°C and heart rate <100 bpm after adequate rest.

Variables

Despite the allotted not allow inferences about causality, for statistical analysis purposes and alignment with the issues of this manuscript the variables measured by skin thermography will be treated as independent variables and mediated percentage expansion brachial artery flow (FMD) as the dependent variable.

Independent variables

The independent variables listed below were generated with the application of thermography infrared radiation (TRIV):

1. Average temperature in the distal phalanges of the fingers of the right hand;

2. Average temperature in the distal phalanges of the left hand's fingers;

3. Basal temperature on the right hand less the minimum temperature in this hand, obtained after 5 minutes of examination (maximum ischemia rate);

4. Maximum temperature obtained between 6 and 15 minutes on the right hand less the maximum ischemia temperature in this hand (reactive hyperemia rate);

5. Maximum temperature on the right hand less the core temperature of the same hand (rebound temperature / RT);

6. Neuro-reactivity: high or average temperature maintenance in the five fingers on his left hand while the right arm occlusion period and drop in temperature in the fingers on his left hand during the closure period of the right arm.

Outcome: Flow-mediated dilatation (FMD) of brachial artery by ultrasound

Measurement of Variables

TRIV - To evaluate patients through thermography use high sensitivity thermal imager infrared sensor Flir® 420, 320 x 240 resolution (76,800 pixels), in the spectral range from far-infrared (8-14 µM) for dynamic study (60 Hz), sensitivity Thermal 0.1°C, adjusted for emissivity of 0.98, which fulfills the necessary characteristics to perform thermographic test with satisfactory quality and follow the protocol for the evaluation of vascular reactivity, as illustrated in (Figure 1). The tests were evaluated by two trained operators in the thermal imaging evaluation and will be blind to the results of the ultrasound of the brachial artery. All patients remained in a room at 25°C and rest for 15 minutes before the test. First registered to thermal images being defined circular areas in the distal phalanges of the five fingers of both hands (ROI) through Tools® dedicated to analysis of thermograms software. The mean daily temperatures basal right and left (step 1). In (step 2) promote supra-systolic pressure of 50 mmHg right arm 5-minute period by inducing ischemia in the distal arm. At this stage, we recorded minimum temperatures of the right hand by getting the maximum temperature of ischemia. Concomitantly we perform measurements of average temperature of the left-hand ROI. The heat records were made every minute.

(Step 3) is the sudden decompression of the right arm causing the blood flow quickly reaches the forearm and the hand. This is the step which is mediated dilatation of the brachial artery flow promoted by the prevailing action of nitric oxide (NO), the most important endogenous vasodilator and relevance in the regulation of endothelial function. At this stage, thermal measures were performed for 10 consecutive

minutes. In thermograms were measured average temperature in the areas marked on the left hand and the maximum temperatures in the right hand.

The tests were evaluated by two physicians trained operators in the evaluation of thermal images. As a rule, put all patients in a room at 25°C, at rest, remains comfortably for 15 minutes before testing.

Thus, recorded thermograms of the palmar aspect of the hand and mark circular areas in the distal phalanges (ROI, region of interest) of the five fingers of both hands through Tools® software getting the average right and left basal temperatures. (step 1)

Following compression performed above 50mmHg systolic right brachial-by period of 5 minutes ischemia in promoting the distal arm. (Step 2) In this step, the minimum temperatures recorded in the right-hand ROI obtaining the maximum temperature of ischemia. (Table.1)

We also recorded at this stage measures of average temperatures in the left hand's ROI. Thermal records from step 2 were made every minute. (Fig.1)

The third step of the assessment is the decompression of the right arm causing the flow of blood reaches the forearm and the hand. In this phase were carried out thermal measurements every minute for 10 consecutive minutes. the average temperature measurements were recorded in the demarcated areas (left hand ROI the maximum temperature of the right-hand ROI).

Table 1 – reactivity test steps by vascular infrared radiation over time.

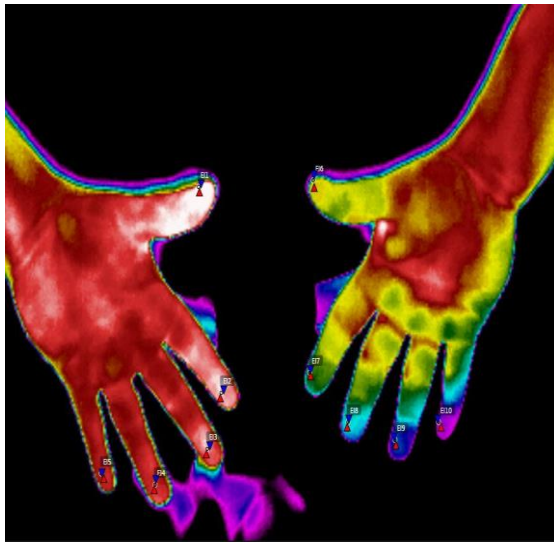
Steps	Right Hand	Left Hand	Time/min
1-basal	T average	T average	0
2-MI	T minimum	T average	5
3-HR	T maximum	T average	6-15

Legend: MI: Maximum ischemia; HR: Hyperemia reactive; T: Temperature

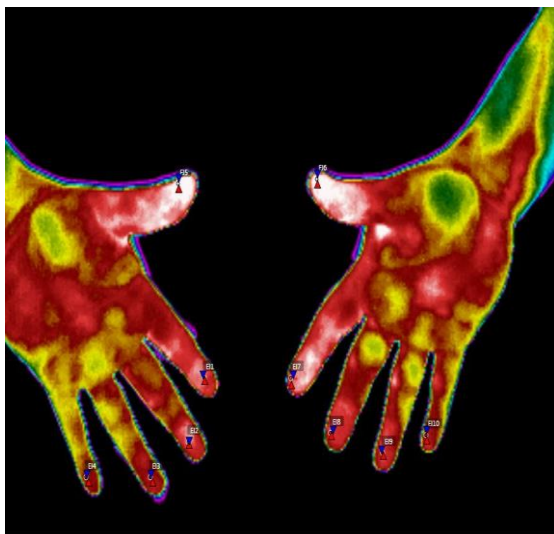
Fig 1. Evolution of the images in relation to the protocol used, respectively at rest (basal stage) during the supra-systolic compression at 2 minutes and 5 minutes (maximum ischemia) and 15 minutes (reactive hyperemia).



(1) Basal 0 minutes



(2) Maximum ischemia at 5 minutes



(3) Reactive Hyperemia

FMD - Measurement of FMD was performed by GE ultrasound equipment - Vivid and linear transducers high frequency (8-11 MHz) for vascular study. The method was performed by an experienced operator in carrying out the method and blind to the thermal results.

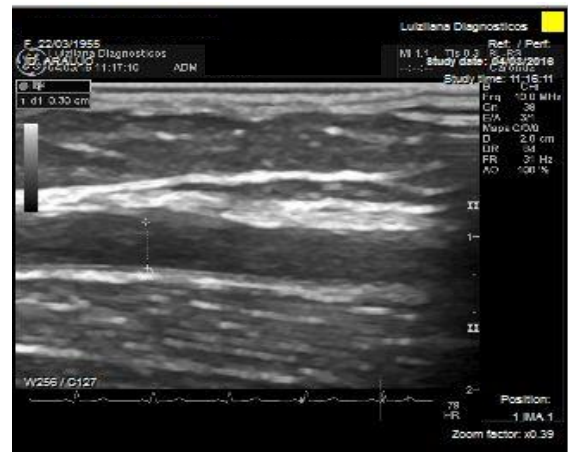
Initially (step 1) is performed to measure the diameter of the brachial artery in longitudinal section between 3 and 5cm from the cubital fossa of the left arm. The measurement is performed on the peak of R concomitant obtained electrocardiogram wave to the ultrasound image. Pen marks were made with hydrocolor able to register the skin exactly the site where supported the transducer into the patient's arm.

(Step 2) Compression with left forearm Tycos® sphygmomanometer of patients with supra-systolic pressure (50mmHg above the value systolic pressure) for 5 minutes.

Measurement of the brachial artery diameter in its longitudinal axis in the same place in which was obtained the initial images, minute by minute until the fifth minute after releasing the compression of the left forearm. (Step 3)

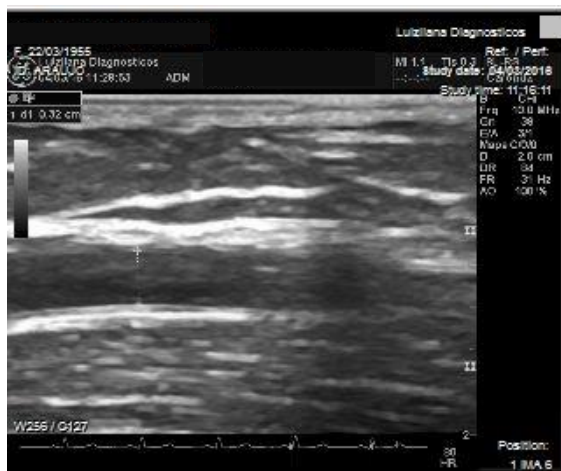
The FMD outcome (%) was thus measured: diameter (mm) of the brachial artery obtained in reactive hyperemia period - initial / 100 diameter.

Fig. 2 Endothelial function using ultrasound examination to check flow mediated dilation of the brachial artery.



The image shows a cross-section of the brachial artery measuring 0.3cm exactly on the R wave of the electrocardiogram (drawn below) prior to compression of the forearm.

Fig 3- Ultrasound image shows the exact moment of flow mediated dilatation



Test Power

It is estimated that the test power is above 80% (type II error, <0.20) for the outcomes: rebound temperature (RT) and neuro reactivity (NR), assuming simple random sampling, and no elements confusion, (type I error = 0.05).

Statistic

We perform in our well-balanced sample (Tables 2 and 3) of patients seeking to bring the real world to our study so we include patients with low, moderate and high cardiovascular risk quantify the relationship between DFM (%) depending on each independent variable, measured by thermography in simple linear regression. The investigation of the waste generated by normal presupposition models adjusted by Shapiro-Wilk and evaluation of the assumption of homoscedasticity waste graphic vs predicted and Spearman correlation analysis to the relations in which the fit of the regression model was not suitable. Comparison subjects rebound temperature (RT) higher and lower than zero compared to FMD (%) by non-parametric Mann-Whitney and Fisher's exact test to buy the degree of FMD to the behavior of neuro reactivity by TRIV. Relationships considered statistically significant if $p < 0.05$. All analyzes were performed using SPSS software (version 15.0; Inc, Chicago, IL, USA).

RESULTS

Table 2 – Sample profile (n = 97)

Women	51 (53%)
Age (years)	60 (24-85)
Hypertension	60 (62%)
Smoker	10 (10%)
Diabetes	21 (22%)
Altered neuro reactivity	24 (25%)

Table 3- Patients classified according to the overall Framingham risk score (FRS) and also with the result of flow-mediated dilatation (FMD) of the brachial artery (low < 5%, Intermediate 6-10% and High > 10%)

	Low	Intermediate	High
FMD	38 (39%)	28 (29%)	31 (32%)
FRS	28 (29%)	33 (34%)	36 (37%)

Table 4- Correlation between thermography variables (TRIV) and the value of Flow mediated dilatation (FMD)

TRIV variables	r (p-value)
Basal temp left hand	$r = -0.0002$ ($p = 0.998$)
Basal temp right hand	$r = -0.01$ ($p = 0.929$)
Rebound temperature	$r = 0.42$ ($p < 0.001$)
Maximum ischemia rate	$r = 0.10$ ($p = 0.322$)
Reactive hyperemia rate	$r = 0.34$ ($p = 0.001$)

r: Spearman's correlation coefficient

The table 4 shows moderate positive correlations but significant between the values of rebound FMD with temperature ($r = 0.52$; $p < 0.001$) and the values of reactive hyperemia rate ($r = 0.34$; $p = 0.001$), indicating that the higher the rebound temperature values and reactive hyperemia rate, the higher the values of the FMD.

DISCUSSION

The behavior of vascular reactivity in the micro-circulation is a little different from large-caliber arteries, not being totally dependent on the endothelium. Central, regional and local mechanisms have a greater or lesser role in the modulation and control of vascular microcirculation tone.⁹

The task of evaluating endothelial function is complex because of the multiple functions performed by this body. Nevertheless, it is a broad understanding that changes in vascular tone after provocative maneuver known as vascular reactivity, translate into much of the action of NO in any vascular bed (macro- and microcirculation). This allows to associate with endothelial health most or lower presence of this autacoid since it participates in a wide range of positive action in order to maintain vascular homeostasis¹⁰.

The result of the study shows a significant association between the FMD and thermography suggesting that the findings obtained by TRIV are associated with endothelial function, however the best combination of thermography with cardiovascular risk score may explain the moderate correlation between tests,

although the two tests have been associated with the Framingham risk score.

It was observed higher prevalence of sympathetic over activity (SO) in individuals with lower FMD can see as shown in Table 5. This result was predictable and initially coincides with previous studies that demonstrated strong association of this variable with the cardiovascular risk because in healthy state sympathetic nerves and vascular endothelial cells share a functional antagonism to maintain vascular tone. In this sense changes in sympathetic activity and function of the endothelial cells which prevent maintenance of this antagonism can be commonly observed since the beginning of the development of cardiovascular disease.¹¹

Table 5 - Prevalence of low, moderate and high risk for stratification accordance with Framingham risk score and neuroreactivity evaluated by TRIV method.

FRS	Normal (n=73)	Abnormal (n=24)
Low risk	28 (38%)	0 (0%)
Interm risk	28 (38%)	5 (21%)
High risk	17 (24%)	19 (79%)

p< 0,001 Fisher's exact test

It was noted that the sympathetic nervous system hyperactivity existence confers moderate to high risk, the more likely a high risk since nearly 80% of those individuals with this SO may be presented risk range. A test with normal neuroreactivity excludes a patient with high cardiovascular risk, unlike what happens with the FMD that despite maintaining good association with the risk of Framingham score, a normal examination was found in 3% of individuals at high risk. (table 6)

Another important aspect to remember is that the RT despite having shown only moderate association with FMD demonstrates significance statistics and is able to clearly verify that adopting randomly a cutoff 0 to RT as shown in Table 7 the ability to discriminate a high value of another low in FMD examination.

Table 6 - Distribution of subjects according to risk ratings by the methods of Flow Mediated Dilation Flow (FMD) and Framingham risk score classification

Table 6 - Framingham Risk score

FMD	Low	Int	High	Total
<5%	20(52%)	14(37%)	4(11%)	38
6-10 %	7(25%)	12(43%)	9(32%)	28
>10%	1(3%)	7(23%)	23(74%)	31

% Concordance = 55/97 (56%); Kappa's Concordance Test = 0,35 (p<0,001)

Table 6 shows a higher prevalence of high cardiovascular risk by FRS among patients with altered neuroreactivity and higher prevalence of low risk among patients with normal neuroreactivity.

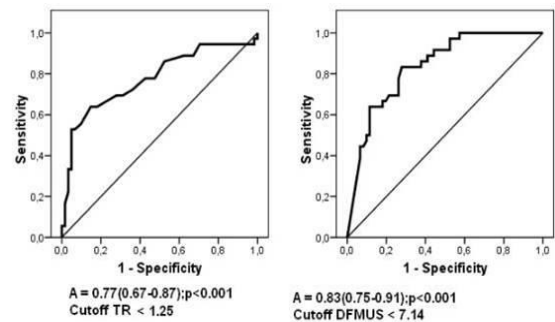
Table 7 - Relationship between rebound temperature and flow mediated dilatation of the brachial artery.

	RT ≥ 0	RT < 0
n =	75	22
Median FMD%	9,6(0,0 - 27,2)	2,8(0,0- 12,10)

p<0,001; Mann-Whitney test).

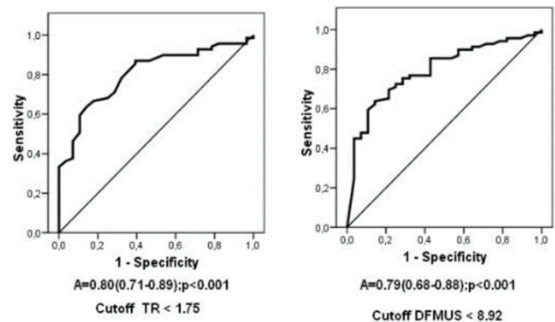
From these data, the TRIV is presented as a tool with potential within the stratification of cardiovascular risk environment and should be better understood since it is noninvasive, not operator dependent, painless and without contrast. However more studies are needed to assess whether this instrument is able to add additional information or reclassify patients with risk scores in use in clinical practice.

Table 8 - We dichotomized the results at high risk of one side and low/moderate risk of another, classified by the GRS / Framingham. In this way, we were able to obtain rebound temperature (RT) values associated with high cardiovascular risk in <1.25 C.



ROC curve above shows a sensitivity of 77% to verify the true positives (high EGR)

Table 9- We separate the results at low risk of one side and moderate/High risk of another, classified by the GRS / Framingham. In this way we were able to obtain rebound temperature (RT) values associated with high cardiovascular risk in >1.75 C



ROC curve above shows a sensitivity of 80% to verify the true positives (low EGR/Framingham)

Our findings are very clear in showing that there is an unequivocal association between microcirculation and macrocirculation behavior and at least in part may be explained by the integrity of endothelial function. Additionally, TRIV offers data on the behavior of the autonomous sympathetic nervous system, which can provide a new field of investigation and make this new form of diagnostic approach even more attractive.

We are conducting larger studies in various scenarios to test for other indications for TRIV beyond stratification of cardiovascular risk.

CONCLUSIONS

Digital infrared thermal imaging associated with provocative maneuver ischemia and reperfusion arm (TRIV) has moderate but significant association with ultrasound flow-mediated dilatation (FMD)

TRIV and FMD have good association with the Framingham/Global Risk Score

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