A novel technique: Indocyanine green angiography to prognosticate healing of foot ulcer in critical limb ischemia.

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Title: A novel technique: Indocyanine green angiography to prognosticate healing of foot ulcer in critical limb ischemia.

Abstract

Background: To predict wound healing in patients with critical limb ischemia (CLI) is an ongoing issue. Current methods like ankle-brachial index (ABI), color doppler and transcutaneous oxygen pressure (TCPO$_2$), CT angiography are lacking in demonstrating regional perfusion. Indocyanine green angiography (ICGA) has the potential to provide regional perfusion information lacking in other methods. To demonstrate successes of revascularization procedure in CLI patients based on ICGA data this study was conducted.

Methods: Total 47 patients with grade 2 or grade 3 UTWCS (University of Texas wound classification system) ischemic foot ulcer undergoing lower limb revascularization procedure were included in this study, from July 2014 to May 2016. ICGA with intravenous 0.1 mg/kg of 0.1% indocyanine green dye was performed pre and post revascularization procedure. ICGA data maximum unit, blush time and blush rate was compared with pre and post revascularization data, along with ABI and TCPO2.

Results: Out of 47 patients (45 males and 2 females) 43 underwent endovascular revascularization and 4 underwent open procedure. Total 76.6% patients were diabetic, 46.8% were hypertensive. 31.9% had coronary artery disease, 21.2% had history of cerebrovascular disease, 23% had chronic kidney disease and 74.4% were chronic smoker. Total 37 patient’s ulcer healed completely on follow-up with significant improvement (‘p’<0.05) in pre and post operative ABI, TCPO2 and ICGA data. Ten patient’s ulcer did not heal in follow-up period. In those 10 patients pre and post operative ABI and TCPO2 improved but ICGA data were not improved post operatively (‘p’>0.05).

Conclusion: ICGA is evolving tool to quantify regional perfusion in CLI. ICGA parameters provide qualitative real time visual images of perfusion in area of interest as well as quantitative information of perfusion.

Keywords: Indocyanine green angiography, prognosticate healing, Non-healing foot ulcer, Critical limb ischemia
INTRODUCTION

Prediction of wound healing in a patient with ischemic limb or diabetic foot is still an unsettled issue. It is a flaming issue for any podiatrist or a vascular surgeon\textsuperscript{[1-2]}. Neuropathy, peripheral arterial disease (PAD), infection, poor glucose control, and compromised skin integrity contributes to an increase in amputation rates (14-43\%) among these population\textsuperscript{[3-5]}.

There are many factors which can contribute to foot ulceration, but a precursor to amputation in majority of cases is the presence of PAD. Underlying PAD is a common finding in individuals with long-standing diabetes, which can negatively affect the healing process of a foot ulcer. Vascular insufficiency alone is not the primary cause of ulceration. Inadequate perfusion can delay or inhibit proper healing of the ulcer by not providing adequate blood flow, leading to further tissue necrosis and the inability to clear infections. The healing rate of a diabetic patient with PAD has been found to be significantly reduced when compared to diabetic patients without underlying PAD\textsuperscript{[1, 5]}. For more than a generation, attempts have been made to measure the perfusion that would be sufficient to ensure wound healing\textsuperscript{[6]}. Many attempts have been made for target area revascularization by angiosome concept to achieve better healing in ischemic foot ulcers\textsuperscript{[7, 8]}.

Current clinical methods of measuring limb perfusion and hemodynamic like ankle-brachial index (ABI), colour doppler and transcutaneous oxygen pressure (TCPO\textsubscript{2}) are often limited by a variety of issues, including the presence of open or infected wounds, or both, previous toe or forefoot infections, and medial calcinosis. These measurements are frequently unable to demonstrate regional perfusion changes, limiting their applicability to many patients with compartmental foot ischemia such as patients with heel ulcers or significant microvascular
A recent meta-analysis suggests the need for further study to establish solid guidelines for the prediction of wound healing as well as to establish whether such studies provide additional, clinically meaningful information beyond that gained from more simply obtained clinical data [9].

Indocyanine green (ICG) is water soluble, non-radioactive and nontoxic contrast agent. It can be visualized with help of laser light source and a charge-coupled camera in the area of interest [6]. Indocyanine green angiography (ICGA) has the potential to provide regional perfusion information which is lacking in standard Doppler derived arterial pressure and waveform measurements. This technology has been used for decades in various clinical areas, and its application is expanding to include assessing viability of skin flaps, measuring myocardium perfusion, and the probability of complications after transplantation [10]. ICGA provides objective, quantifiable, and reproducible parameters of perfusion [6, 11], that aids in the clinical decision to determine the need for revascularization, success of revascularization procedures and in directing wound management and closure. Thus we aimed to study the success of revascularization procedures based on ICGA data.
METHODS

A study of 51 patients with grade 2 or grade 3 UTWCS (University of Texas Wound Classification System) ischemic foot ulcer patients who were undergoing lower limb revascularization procedure was conducted in the department of peripheral vascular and endovascular surgery at Medanta the Medicity hospital, Gurgaon, India, from July 2014 to May 2016. All patients with Age < 18 years and > 80 years, Poor liver function, massive edema of foot, active cellulites with foot ulcer, non salvageable limb and history of allergic reaction to contrast media were excluded from the study. After taking informed and written consent, all patients underwent Doppler scanning, ABI, TCPO2, Clinical photography of ulcer and ICGA one day before the revascularization procedure after thorough cleaning of ulcer and 24hrs after the revascularization procedure.

The SPY fluorescent (Novadaq) imaging system (Image 1) consists of an imaging head equipped with a charged coupled device (CCD) camera, a laser light source and a distance sensor; was used for ICGA. After removing all the dressing from the foot, patients were given proper position. Once the camera was in place, 0.1 mg/kg of 0.1% ICG (indocyanine green) solution was administrated intravenously with bolus infusion followed by a push of 10 ml normal saline to flush the intravenous route, simultaneously the room lights were dimmed. Images were taken with camera. The areas of fluorescence intensity was viewed in gray-scale, white area was indicative of higher intensity; while in a heat-map mode red was indicative of high intensity and blue area was indicative of low intensity of perfusion in the area of interest / foot ulcer (as shown in image 2). Percentage of perfusion of ischemic ulcer as well as other parameter like maximum unit (intensity increase from baseline to peak), blush time (time taken for increase in intensity
from baseline to peak intensity), blush rate (rate of intensity increase from baseline to peak intensity over time) (Image 3) was recorded before and after revascularization procedure.

Local wound care depended on the characteristics of each ulcer. Standard surgical protocol for wound management depending on character of wound was followed (for example, Debridement, wound microbiology culture, wound closure methods, vacuum assisted dressing etc.). Wound healing was defined as complete epithelialisation of the tissue defect. Follow up of patients was taken on 15 ± 2 days, 30 ± 4 days and 60 ± 7 days after discharge and further follow up was done depending on the status of wound, maximum up to 180 ± 7 days.

Statistical analysis included profiling of patients on different demographic, clinical and biochemical parameters. Comparison of healed cases with others was presented with respect to prognostic factors. Descriptive analysis of quantitative parameters was expressed as mean and standard deviation. Ordinal data was expressed as percentage. The analysis was Univariate as well as multivariate. Student t test, z test and chi-square test were used to compare the data between the pre operative and post operative groups. P-value < 0.05 was considered statistically significant. All analysis was done using IBM SPSS statistics 24.
RESULTS

A total of 51 patients were enrolled in this study, out of which 4 patients were excluded from the study due to altered liver function tests (n=1) and active cellulites of ulcer (n=3). Hence, a total of 47 patients (45 males and 2 females) were included in the study from July 2014 to May 2016. Mean age of the patients was 59.84 ± 10.84 years. The co-morbidities like diabetes, hypertension, chronic kidney disease, coronary artery disease, cerebrovascular disease significantly affects outcome in these patients. Total 36 patients (76.6%) were diabetic, 23 patients (46.8%) were hypertensive. Fifteen patients (31.9%) had coronary artery disease, 21.2% patients had history of cerebrovascular disease. Twenty-three percent of patients had chronic kidney disease and 74.4% patients were addicted to smoking tobacco. Forty-three out of 47 patients underwent endovascular procedure and 4 patients underwent open distal bypass surgery.

The highest value of pre operative ABI was 0.56 and lowest was 0.0 (non recordable), and 1.3 and 0.0 post revascularization respectively. Total of 8 patients’ out of 47 had an ABI that was non recordable at pre and post revascularization procedure. As per ICGA data, highest value of percentage of perfusion of wound was 67% and lowest was 14% before revascularization procedure and 91% and 16% post procedure respectively. Before the revascularization procedure the maximum unit of perfusion of ulcer was noted between 1 unit and 150 units, and post procedure it was between 1 unit and 234 units. Before revascularization procedure maximum time taken to reach maximum unit was 55.4 seconds (s) and minimum was 0.2 s and after revascularization it was 46.9 s and 0.3 s respectively. Highest value of blush rate was 11.9 unit/s and lowest was 0.1 unit/s before revascularization, and 63 unit/s and 0.1 unit/s after revascularization respectively. In the present study wound was considered as non-healing if it did not heal after 180 ± 7 days. Maximum follow up period post procedure was 184 days with mean follow up being 82.1 ± 60.5 days. There were 37 patients whose ulcers healed completely and the mean follow up of this group was 55.69 ± 35.9 days. Ulcer did not heal in 10 patients, and the mean follow up of this group was 180 ± 2.6 days.
Out of 47 patients studied there were 37 patients whose ulcer healed completely during the follow up period. Among this group of patients the pre-operative mean ABI was 0.3524, which significantly increased after revascularization to 0.8284, with a highly significant ‘p’ value (p < 0.0001). Pre-operative mean of TCPO2 was 30.92, which increased after revascularization with a significant ‘p’ value (<0.0001) (Table 1). Maximum unit also improved significantly after revascularization procedure with a ‘p’ value of less than 0.0001. Preoperative mean blush rate was 2.287 units/s, while after revascularization the mean blush rate was 7.144 units/s and this difference was statistically significant (p < 0.05). However the ‘p’ value for blush time was not significant. ABI and TCPO2 values were correlated with ICGA parameters by Pearson’s correlation coefficient and are shown in Table 2.

In the group of 37 patients with healed ulcer, 6 patients’ ABI were not recordable even after revascularization; but there was a statistically significant difference in the TCPO2 values in these 6 patients (p < 0.01). Similarly, in those 6 patients even though the ABI was not recordable the mean maximum unit and blush rate (ICGA parameters) showed improvement after revascularization with a statistically significant ‘p’ value (p < 0.05) (Table 3).

There were no signs of healing of ulcer in 10 patients out of 47 even after 180+/−7 days follow up. Even though ABI and TCPO2 were improved significantly after revascularization (‘p’<0.05), maximum unit, blush time and blush rate was not improved even after revascularization (‘p’ value >0.05) (Table 4).
DISCUSSION

There are many methods available today to evaluate the perfusion in critical limb ischemia like ABI, Toe pressure, TCPO2, colour doppler, computer tomography scan and magnetic resonance imaging. But all of these investigations lack in providing specific ulcer perfusion information that can directly provide information regarding success of revascularization or can correctly predict the ulcer healing in critical limb ischemia. These methods will provide vascular structural information only, without vascular functional level at area of interest in critical limb ischemia. Functional perfusion information in case of vascular insufficiency is much superior to vascular structural information\[11, 13\].

Indocyanine green angiography (ICGA) has potential to provide regional quantitative as well as qualitative visual information of ulcer perfusion. ICGA is being used since a long time in ophthalmology, during liver transplant, coronary artery bypass and plastic reconstructive surgical procedures. Many attempts have been made for its use in critical limb ischemia to measure perfusion of limb as well as to predict outcome of wound\[6, 11, 14\]. ICGA will provide quantitative data that can be quickly and reliably derived from the images to determine the degree of tissue perfusion, to evaluate the therapeutic effects of revascularization procedures and need of further intervention. Braun et al\[6\] in their study found that ICGA can provide significant information based on its ICGA data regarding the outcome of revascularization. Naoki U et al\[14\] reported successful use of ICGA after paramalleolar artery bypass using saphenous vein grafts to check patency and distal perfusion. Kang et al\[15\] reported the use of ICGA parameter like maximum intensity to diagnose functional vascular insufficiency in patients with Raynaud’s phenomenon.

To minimize the error during ICGA standard protocol was followed as described by Braun et al\[6\] study. Small deviation in this protocol may lead to change in the quantitative data.
and visual images in ICGA. There is no standardized value or criteria defined based on previous
ICGA study to identify critical limb ischemia (CLI). CLI is defined as an ankle systolic pressure
of 50 mm Hg or less and with or without non healing wound or gangrene. The study of
Kimihiro Igari et al \cite{11} predicted CLI by a T1/2 (time elapsed from fluorescence onset to half the
maximum intensity) value more than 20 seconds. In the present study all patients presented with
non healing ulcers the pre-operative ABI was ≤0.5. In the present study, mean maximum unit
was 22.02 ± 32.2 units , which was 29.7 described as ‘Ingress’ by Braun et al \cite{6} , and 23.3
described as ‘Imax’ by Kimihiro Igari et al \cite{11}; which are comparable. In both these studies the
‘p’ value was significant for maximum unit when compared with pre and post operative values;
similarly in the present study also the ‘p’ value was <0.05 for this parameter. Mean blush rate
was 2.28 ± 4.6 units/s preoperatively which was comparable with the above two studies \cite{6, 11}.
Based on ICGA data of the present study we could categorize patients as having CLI if
maximum unit is ≤ 22.02 ± 32.2 units and blush rate is ≤ 2.28 ± 4.6 units/s for the foot ulcer;
however a larger scale study is needed to validate such a criteria.

With use of a definitive protocol for ICGA very useful parameters like perfusion
percentage, maximum unit (intensity increase from baseline to peak), blush time (time taken for
increase in intensity from baseline to peak intensity), blush rate (rate of intensity increase from
baseline to peak intensity over time) can be obtained from the areas of interest of an ulcer in
patients with critical limb ischemia. From these parameters useful information regarding the
perfusion of ulcer and improvement in perfusion after revascularization procedure can be
obtained.

ABI, TCPO2, CT angiography, colour doppler have many limitations in presence of open
or infected wounds, previous toe or forefoot infections, medial calcinosis of vessel wall. As
stated before they are lacking in providing regional perfusion which is very important in case of non healing ulcers in CLI patients. ICGA has potential to provide regional perfusion information which is lacking in standard Doppler derived arterial pressure and waveform measurements. As we can see in Image 4 patient had non-healing ulcer over plantar surface of foot. Preoperative ICGA showed that there was very poor perfusion in ulcer area on gray scale as well as colour scale. On graphical picture preoperative maximum unit was 5 units, blush rate was 0.2 unit/s and blush time was 23.2 s which significantly improved after below knee angioplasty and was 55 units, 3.9 units/s and 12.7 s respectively (Image 5).

In the present study, out of 47 patients, ulcers of 10 patients did not heal. Although in these 10 patients ABI and TCPO2 improved with a significant ‘p’ value (p < 0.05) after revascularization, the ICGA parameters showed no improvement (p > 0.05). This is probably due to poor collateral circulation across the choke vessels of the foot. In the present study ICGA parameters were more reliable in predicting the outcome of ulcer healing after revascularization, than other parameters. In 37 patients out of 45 ulcers healed completely. ICGA parameters significantly improved after revascularization with a statistically significant ‘p’ value (p < 0.05) in these 37 patients. In total 6 patients out of 37 patients ABI was non-recordable after revascularization procedure, but maximum unit and blush rate was improved in these patients after revascularization with statistically significant ‘p’ value (‘p’ <0.05). Although the study population was small in the present study, we can still conclude that approximate 41.51 ± 31.4 unit increase in maximum unit and 5.31 ± 10.32 unit/s increase in blush rate can predict ulcer healing in non-healing foot ulcer after revascularization procedure.

ICGA is not limited by the medical calcinosis of vessels; it will provide accurate result of perfusion up to the depth of 5 to 6 mm in the area of interest in the non healing ulcer, which is
lacking in the other methods. However in case of acute inflammation or acute cellulitis of ulcer it is not advisable to perform ICGA as it may give false positive result. ICGA will give quantitative parameters of perfusion as well as qualitative visual image of perfusion of the area of interest. Qualitative visual images of different areas of interest in the same limb can be obtained at the same time.

We believe that this was the first attempt to measure the perfusion of the non-healing ulcer in CLI patients with ICGA in India. Although the sample size was small in the present study, there were some interesting findings at the end of study which can be helpful for future studies. In future ICGA can be very useful for evaluation of - non-healing wounds, wounds with delayed closure, readiness of wound for skin grafting, amount of tissue debridement, etc. It might be useful for defining new criteria and grading for CLI.

Indocyanine green is completely metabolized in liver and has complete hepatic clearance. It is a non-toxic dye and has a very low incidence of adverse reactions of only 4 out of 240,000 doses [5]. It can be very safely used in patients with chronic kidney disease without any adverse effects. It can be safely used as both intravenous as well as intra arterial route; in the present study intravenous route was used. It is very easily performed on OPD basis or at bed side. It is strongly recommended that patients with history of allergy to iodine, iodine product, contrast media should avoid undergoing Indocyanine green dye based investigations. In the present study there was no adverse reaction to Indocyanine green.
CONCLUSION

ICGA is an evolving tool to quantify regional perfusion in CLI patients. ICGA parameters provide qualitative real time visual images of perfusion in area of interest as well as quantitative information of perfusion. It is valuable to evaluate the success of revascularization in CLI patients. Based on the present study we conclude that, an increase in maximum unit up to 41.51 ± 31.4 units with increase in blush rate up to 5.31 ± 10.32 units/s can predict ulcer healing in non-healing foot ulcer in CLI patients after revascularization procedure.
References:


**Image 1:**
Novadaq Spy (Indocyanine green angiography) angiography machine.

**Image 2:**
Image showing the gray and color scale of foot perfusion on ICGA before and after revascularization procedure.

**Image 3:**
ICGA parameters in graphical view (maximum unit, blush time and blush rate).

**Image 4:**
Pre operative ICGA gray scale and color scale, arrow showing poor perfusion in ulcer area and in all toes. Post operative ICGA, arrow showing improved perfusion in ulcer area in gray and color scale.

**Image 5:**
Pre and Post operative Graphical representation of perfusion on ICGA showing perfusion parameters (maximum unit, blush time and blush rate) of same patient shown in Image 4.
Table 1: Statistical analysis of ABI, TCPO2, Maximum unit, Blush time and Blush rate of patients with healed ulcer.

<table>
<thead>
<tr>
<th>Group</th>
<th>ABI Pre-operative</th>
<th>ABI Post-operative</th>
<th>TCPO2 Pre-operative</th>
<th>TCPO2 Post-operative</th>
<th>Maximum Unit Pre-operative</th>
<th>Maximum Unit Post-operative</th>
<th>Blush Time Pre-operative</th>
<th>Blush Time Post-operative</th>
<th>Blush Rate Pre-operative</th>
<th>Blush Rate Post-operative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.3524</td>
<td>0.8284</td>
<td>30.92</td>
<td>48.57</td>
<td>22.84</td>
<td>63.43</td>
<td>19.259</td>
<td>18.157</td>
<td>2.287</td>
<td>7.144</td>
</tr>
<tr>
<td>SD</td>
<td>0.2353</td>
<td>0.4048</td>
<td>18.69</td>
<td>20.46</td>
<td>33.43</td>
<td>46.66</td>
<td>14.833</td>
<td>11.466</td>
<td>4.747</td>
<td>13.1788</td>
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<tr>
<td>N</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
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<td>37</td>
<td>37</td>
<td>37</td>
<td>37</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.65</td>
<td>0.008</td>
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(SD= Standard Deviation, N=Number of patients)
Table 2: Correlation of Ankle-Brachial pressure Index (ABI) with Indocyanine green angiography (ICGA) and TCPO2 with ICGA parameters by Pearson correlation coefficient of patients with healed ulcer.

<table>
<thead>
<tr>
<th>Correlation with ABI</th>
<th>No</th>
<th>Percentage perfusion of ulcer</th>
<th>Maximum unit</th>
<th>Blush time</th>
<th>Blush rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>37</td>
<td>0.165</td>
<td>-0.031</td>
<td>0.135</td>
<td>0.067</td>
</tr>
<tr>
<td>$r^2$</td>
<td>37</td>
<td>0.027</td>
<td>0.001</td>
<td>0.018</td>
<td>0.004</td>
</tr>
<tr>
<td>P</td>
<td>37</td>
<td>0.329</td>
<td>0.86</td>
<td>0.44</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Correlation with TCPO2

<table>
<thead>
<tr>
<th>R</th>
<th>37</th>
<th>0.035</th>
<th>-0.01</th>
<th>0.21</th>
<th>-0.08</th>
</tr>
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<tr>
<td>$r^2$</td>
<td>37</td>
<td>0.0013</td>
<td>0.0001</td>
<td>0.046</td>
<td>0.0077</td>
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<tr>
<td>p</td>
<td>37</td>
<td>0.77</td>
<td>0.69</td>
<td>0.08</td>
<td>0.63</td>
</tr>
</tbody>
</table>

(R= correlation coefficient R, $r^2$= coefficient of determination, p= probability) (The nearer the value of R to zero, the weaker the relationship)
Table 3: Statistical analysis of ABI, TCPO2, Maximum unit, Blush time and Blush rate of patients in whom ABI was Non-recordable after revascularization with healed ulcer.

<table>
<thead>
<tr>
<th>Group</th>
<th>ABI Pre-operative</th>
<th>ABI Post-operative</th>
<th>TCPO2 Pre-operative</th>
<th>TCPO2 Post-operative</th>
<th>Maximum Unit Pre-Operative</th>
<th>Maximum Unit Post-Operative</th>
<th>Blush Time Pre-Operative</th>
<th>Blush Time Post-Operative</th>
<th>Blush Rate Pre-Operative</th>
<th>Blush Rate Post-Operative</th>
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<tr>
<td>MEAN</td>
<td>0</td>
<td>0</td>
<td>30.00</td>
<td>38.00</td>
<td>25.17</td>
<td>62.83</td>
<td>15.750</td>
<td>12.983</td>
<td>1.250</td>
<td>5.583</td>
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<tr>
<td>SD</td>
<td>0</td>
<td>0</td>
<td>5.93</td>
<td>10.12</td>
<td>41.92</td>
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<td>1.407</td>
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</table>

(SD= Standard Deviation, N=Number of patients)
Table 4: Statistical analysis of ABI, TCPO2, Maximum unit, Blush time and Blush rate of patients with non-healing ulcer.

<table>
<thead>
<tr>
<th>Group</th>
<th>ABI Pre-operative</th>
<th>ABI Post-operative</th>
<th>TCPO2 Pre-operative</th>
<th>TCPO2 Post-operative</th>
<th>Maximum Unit Pre-Operative</th>
<th>Maximum Unit Post-Operative</th>
<th>Blush Time Pre-Operative</th>
<th>Blush Time Post-Operative</th>
<th>Blush Rate Pre-Operative</th>
<th>Blush Rate Post-Operative</th>
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<tbody>
<tr>
<td>Mean</td>
<td>0.3940</td>
<td>0.7460</td>
<td>29.70</td>
<td>38.40</td>
<td>19.00</td>
<td>21.20</td>
<td>18.230</td>
<td>15.780</td>
<td>2.2790</td>
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<tr>
<td>SD</td>
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<td>0.4180</td>
<td>10.38</td>
<td>14.98</td>
<td>28.88</td>
<td>31.77</td>
<td>19.385</td>
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<td>4.2902</td>
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<tr>
<td>P value</td>
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<td>0.02</td>
<td>0.10</td>
<td>0.59</td>
<td>0.41</td>
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(SD= Standard Deviation, N=Number of patients)