Venous duplex and pathologic differences in thrombus characteristics between de novo deep vein thrombi and endovenous heat-induced thrombi

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Objective: Superficial venous reflux disease has been treated with endovenous ablation techniques for more than 15 years. Thrombi discovered in the postoperative period are referred to as endovenous heat-induced thrombi (EHIT). In spite of the few studies of the ultrasound differentiation between EHIT and deep vein thrombi (DVT), there remains a paucity of literature regarding the evaluation of ultrasound examination and pathologic differentiation.

Methods: Six Yorkshire cross swine underwent femoral vein thrombosis by suture ligation or endovenous radiofrequency ablation. At 1 week after the procedure, each femoral vein was imaged by color Duplex ultrasound and sent for histologic interpretation for differentiation between EHIT and DVT. Five blinded vascular surgery faculty, two vascular surgery fellows, and three vascular surgery residents reviewed the ultrasound images.

Results: Thrombi associated with radiofrequency ablation demonstrated a greater degree of hypercellular response, fibroblastic reaction, and edema (3.42 vs 2.92; 3.75 vs 2.42; 2.83 vs 1.33). Specimens harvested from the iatrogenic-induced DVT swine demonstrated a more prolife response to trichrome staining (3.42 vs 2.67). Evidence of revascularization was found in all of the EHIT specimens but in 33% of DVT specimens. On the basis of histologic findings, the pathologist predicted correct modality 92% of the time. Subgroup analysis comparing paired specimens from each swine failed to demonstrate any marked pathologic differences. Recorded ultrasound images from EHIT and DVT samples were reviewed by fellows, residents, and vascular surgery staff to determine whether clot was stationary or free-floating (n = 111; 93%), evidence of retracted or adherent vein (n = 105; 88%), and absence of color flow (n = 102; 85%). The degree of occlusion (partial vs total) and degree of distention of a visualized vein were least likely to be agreed on by reviewers (n = 95; 79% each, respectively). In subgroup (DVT vs EHIT) analyses, the percentage agreement was greatest among vascular surgery fellows (89% and 92%) compared with residents (82% and 79%) and faculty (78% and 77%).

Conclusions: It is possible to differentiate the thrombus origin on pathologic examination but not clinically on ultrasound. Wide variability exists for ultrasound diagnosis of EHIT and de novo DVT. Care must be taken in evaluating post-treatment duplex scans to not assign diagnosis of EHIT when DVT may well be present and extending into the deep venous system. The modulation of collagen production in the treatment of DVT may be helpful in preventing vascular dysfunction and reducing the post-thrombotic changes. Further studies on injury after radiofrequency ablation and laser ablation are needed. (J Vasc Surg: Venous and Lym Dis 2014;1:1-6.)

Clinical Relevance: Care must be taken in evaluating post-treatment duplex scans to not assign a diagnosis of EHIT when DVT may well be present and extending into the deep venous system. Use of color Duplex ultrasound to establish a diagnosis of either DVT or EHIT may prove to be helpful in differentiating the most appropriate treatment for patients.

Varicose veins and venous reflux have been problem issues for both patients and those caring for them for more than half a century. Vein stripping used to be the method of choice for treatment of these issues. However, the first method using electrocoagulation to treat lower extremity venous varices was reported in 1964.1 The processes and techniques used to treat venous insufficiency have subsequently undergone, like many procedures in the surgical realm, extensive modification and adaptations.2,7 The most widely adopted approach, since 1997, involves endovenous techniques to treat superficial venous reflux disease.8,9 The reported benefits of these techniques include less discomfort for the patient and earlier return of patients to activities of daily living.10
The complication rate of this procedure is reported to be 1% to 16%.\textsuperscript{7,11} Whereas most complications are often related to bruising and self-limited paresthesias, there have been reports of complications as devastating as the development of an arteriovenous fistula with progression into high-output cardiac failure.\textsuperscript{12} Thrombi that are found in the postoperative period after endovenous radiofrequency ablation (RFA), whether by radiofrequency or laser, are referred to as endovenous heat-induced thrombi (EHIT).\textsuperscript{13} EHIT development in the postoperative period can be of concern, especially if the thrombus extends into the deep femoral venous system. Treatment of these sequelae remains controversial. Some authors suggest anticoagulation therapy for EHIT, whereas others have outlined various treatment algorithms based on the extent of thrombus extension into the deep femoral vein.\textsuperscript{14,15} The clinical and treatment considerations for EHIT are not well defined in the literature despite the vast number of studies related to deep vein thrombi (DVT) that are not associated with endovenous ablation techniques. In spite of the few studies of the ultrasound differentiation between EHIT and DVT, there remains a paucity of literature regarding the evaluation of the ultrasound examination and the pathologic differentiation.\textsuperscript{14–17} Although studies have evaluated the prevalence of EHIT and proposed treatment regimens, they have failed to verify and to validate the ultrasound characteristics of EHIT.\textsuperscript{14,16} It would seem plausible that the pathologic inciting event for the development of a de novo DVT would potentially be different from that of an EHIT. This could potentially create a dichotomy of treatment implications as well as future surveillance strategies.

The objective of the study was to assess the ability to differentiate EHIT and DVT with regard to pathologic and ultrasound evaluations among vascular surgery faculty, vascular surgery fellows, and vascular surgery residents.

**Methods**

Six Yorkshire cross swine were placed under general anesthesia and underwent femoral vein thrombosis by either suture ligation or endovenous RFA. Bilateral femoral vein exposures were performed by standard knife and electrocautery dissection. Each femoral vein was either suture ligated with a 2-0 silk suture or ablated by an RFA catheter (Covidien, Mansfield, Mass) that was inserted through a venotomy made distally in the femoral vein. The radiofrequency catheter was activated twice in the same location, akin to the technique endorsed for human use at the most proximal ablation site. Tumescent anesthesia was not used during the procedure as the exposure was open and not percutaneous. Once the vein was suture ligated or ablated, the incision was closed in standard three-layer technique with skin edges reapproximated by a 4-0 Monocryl suture. Each animal had the same procedure (suture ligation or RFA) on each femoral vein and was subsequently extubated.

At 1 week after the procedure, each swine was placed under general anesthesia, and each femoral vein was imaged by color Duplex ultrasound and recorded for review and analysis by the vascular surgery residents, fellows, and faculty. The femoral vein was subsequently harvested, and the source of all specimens was de-identified for a review by a single pathologist. Histologic review used both hematoxylin and cosin stain and trichrome stain. A pathologist evaluated the thrombi for evidence of hypercellular and fibroblastic reaction, presence of neovascularization, edema, and age of clot. Each characteristic was graded...
on a scale of 0 to 5, and the severity or intensity of the finding was correlated with a higher number. The pathologist predicted the etiology of each thrombosis as DVT or EHIT.

The recorded Duplex ultrasound scans were de-identified and reviewed by a combination of vascular surgery faculty (n = 5), 5+2 fellows (n = 2), and 0+5 vascular residents (n = 3). Each reviewer classified the visualized thrombus into 10 categories including echogenicity, clot characteristics, degree of occlusion, presence of color flow, and age of clot.

Animals were housed in the animal barrier facility at Good Samaritan Hospital (Cincinnati, Ohio). Animal care was provided according to the Hatton Institute Animal Care protocol, which is in compliance with the National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals. All research was approved by the Good Samaritan Hospital Institutional Animal Care and Use Committee and Institutional Review Board.

RESULTS

Histologic examination revealed acute or subacute thrombi in each femoral vein specimen. Thrombi associated with RFA demonstrated a greater degree of hypercellular response, fibroblastic reaction, and edema (3.42 vs 2.92; 3.75 vs 2.42; 2.83 vs 1.33). Evidence of vascular reaction with relation to the severity of cell proliferation was equal between the two groups (Fig 1). Specimens harvested from the iatrogenic-induced DVT swine demonstrated a more prolific and cellular dense response to trichrome staining (3.42 vs 2.67) (Figs 2 and 3). Evidence of neovascularization was found in all of the EHIT specimens and in only 33% of DVT specimens. There was no evidence of recanalization in the specimens. On the basis of the histologic findings, the pathologist predicted the correct modality 92% of the time. Subgroup analysis comparing paired specimens from each swine failed to demonstrate any marked pathologic difference.

Review of the recorded ultrasound examinations revealed that the most commonly agreed on duplex findings in both groups were presence of stationary or free-floating clot (DVT, 95%; EHIT, 90%), evidence of a retracted or adherent vein (DVT, 92%; EHIT, 84%), and absence of color flow (DVT, 76%; EHIT, 94%). The echogenicity (bright vs faint) and the appearance of the clot as smooth or irregular of each specimen were each corroborated by 81% of reviewers. The degree of compressibility of the thrombosed vein was agreed on by 84% of reviewers. The degree of occlusion (partial vs total) and degree of distention of a visualized vein were the least likely to be agreed on by reviewers (79% each, respectively). There was no difference in percentage agreement between the EHIT and DVT groups compared against the various groups of reviewers (Table I).

In subgroup (DVT vs EHIT) analyses, the percentage agreement was greatest among the vascular surgery fellows compared with residents and faculty (Table II).

The findings of the reviewers were relatively similar between the two groups in a few categories, including how

Table I. Percentage agreement among duplex ultrasound examination reviewers

<table>
<thead>
<tr>
<th>Feature</th>
<th>DVT, %</th>
<th>EHIT, %</th>
<th>Overall, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degree occlusion</td>
<td>73</td>
<td>84</td>
<td>79</td>
</tr>
<tr>
<td>Free-floating</td>
<td>95</td>
<td>90</td>
<td>93</td>
</tr>
<tr>
<td>Retraction</td>
<td>92</td>
<td>84</td>
<td>88</td>
</tr>
<tr>
<td>Distention</td>
<td>75</td>
<td>83</td>
<td>79</td>
</tr>
<tr>
<td>Compressibility</td>
<td>82</td>
<td>86</td>
<td>84</td>
</tr>
<tr>
<td>Character</td>
<td>87</td>
<td>75</td>
<td>81</td>
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<tr>
<td>Echogenicity</td>
<td>84</td>
<td>78</td>
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</tr>
<tr>
<td>Homogenicity</td>
<td>89</td>
<td>70</td>
<td>80</td>
</tr>
<tr>
<td>Color flow</td>
<td>76</td>
<td>94</td>
<td>85</td>
</tr>
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DVT, Deep vein thrombi; EHIT, endovenous heat-induced thrombi.
adherent or retracted the thrombus was with regard to the wall of the vein as well as the degree of distention or contraction of the vein (Table III). Thirty-two percent of the reviewers rated the DVT and EHIT as firm, whereas 68% believed the thrombi were soft. Nearly two thirds of each group was characterized as being smooth in appearance, but 22% of DVT and 31% of EHIT were irregular in appearance. The clot was stationary in the majority of each group (95% DVT, 89% EHIT). Whereas 73% of EHIT appeared to be associated with total occlusion of the vessel on ultrasound examination, only 41% of DVT were rated similarly. Also in contrast to the bright appearance on ultrasound imaging of exactly two thirds of EHIT, only 38% of DVT were rated the same. Last, there was a greater likelihood of absent color flow in the EHIT group (95%) compared with the DVT group (60%).

**DISCUSSION**

We found a few consistent differentiating results on histologic examination between DVT and EHIT in this study. Iatrogenic DVT was more often associated with a more intense reaction on trichrome stain when viewed under the microscope. Trichrome stain in this setting is a marker of collagen production. This same level of collagen reaction has previously been demonstrated in mouse models. The clinical significance of this increased collagen production could be the foundation on which the vein wall remodeling process occurs. Interestingly, whereas the EHIT specimens demonstrated increased fibroblastic reaction, which tends to be associated with increased scar tissue formation, the level of reactivity to the trichrome stain was much less. What would at first glance appear to be contrary to this finding is the previous report of the mechanism of action of RFA, in which we describe RFA closure inducing vein wall collagen contraction through a denaturation process. Previous findings have suggested that EHIT is formed by collagen being denatured, and thus it would have been predicted to be more prevalent in this group. Contrary to this, new collagen production was found to be greater in the DVT group. This differentiation between the two groups could, in the future, serve as the goal of treatment to target inhibition of collagen production in DVTs and potentially help lessen the incidence of postphlebitic syndrome and venous insufficiency in patients affected by DVT.

The presence of a vascular reaction or proliferation around the specimen was virtually identical between the two groups, and the EHIT group exhibited a more elevated hypercellular response. These findings, although interesting to examine and to discuss, have led the authors to appreciate the vast number of variables within this field of study for which explanations are still not available, and further study is needed.

The increased incidence of edema found on the histologic slides of the EHIT specimens is supported by the thermal spread of the ablation technique and natural response to this type of thermal energy. This coincides with the previously accepted technique of injecting tumescent solution above the vein to be ablated to help prevent the thermal spread (“heat sink”) from affecting the more superficial layers of the dermis and surrounding tissues.

The results suggest that histologic examination can accurately differentiate DVT from EHIT 92% of the time. This carries possible implications in discovering the etiology for mortality in patients after RFA and sudden death.

Although it is challenging if not impossible to obtain statistically significant numerical results with a small study size such as this, the histologic results between each paired group of specimens (Fig 1) do provide some level of certainty that there were no outliers among the two groups. In the initial design of the study, we had considered inducing an iatrogenic DVT on one side and an EHIT on the opposite side of each swine. However, the rationalization to induce the
same type of thrombus in each swine was so that we would have the ability to provide consistent results among the two separate groups and avoid any potential systemic variation induced by one technique on the other.

The comparison analysis of ultrasound interpretation by blinded reviewers suggests that there is significant variability in characterizing acute thrombi. Our reviewers comprised vascular surgery residents enrolled in a 0+5 vascular residency program, vascular surgery fellows enrolled in a 5+2 fellowship, and vascular surgery faculty. Whereas the vascular fellows had previously completed a curriculum of vascular ultrasound interpretation and currently interpret the noninvasive vascular laboratory studies, the vascular residents’ education dedicated to ultrasound interpretation was limited. Therefore, a manual of the basics in vascular ultrasound interpretation was provided to each resident before reading of the blinded ultrasound images of the study specimens. This is, of course, a limiting factor in the assessment; however, the results of the blinded interpretations have been maintained in separate categories to allow delineation of results along these lines.

Limitations to this study, in addition to the variable experience of ultrasound interpretation of the groups, were the small sample size of specimens and the single-institution evaluation. Whereas the consistency in histologic analysis by a single pathologist might be considered a strength of the study, it is certainly noteworthy to be mentioned as a potential limitation as well.

Vascular surgery fellows tended to have a greater degree of agreement in defining clots, which may be related to the increased volume of study interpretations completed during this focused training period.

It has been well established that the more chronic a clot is, the harder it is, and thus the less compressible the vein is on ultrasound examination. Among all of the reviewers, 84% were in agreement in regard to the compressibility of the thrombus on examination. This correlated with the finding that less than a third of the reviewers rated the thrombi, in both groups, as firm vs soft.

Differentiating characteristics on ultrasound examination between the two groups were varied. The most impressive differences were noted to be related to the echogenicity of the thrombi. Those associated with EHIT were brighter on imaging vs the more faint characteristics of the DVT group. This could be correlated to the increased fibroblastic reaction exhibited on pathologic examination as well.

If the common femoral vein is either sutured closed or ablated with radiofrequency energy, the entire vein would, in theory, become occluded, and no flow would be demonstrated through the segment distal to this site; however, this was not necessarily the case in this study. The presence or absence of color flow on ultrasound imaging was also more varied. Thrombi associated with EHIT were more likely to completely occlude the vessel lumen and subsequently prevent any color flow on ultrasound imaging.

Unfortunately, there is no ultrasound criterion that was able to accurately differentiate the etiology of the thrombus. Clinical implications for management result in all thrombi being treated on the basis of thrombus extent through the saphenofemoral junction.

CONCLUSIONS

It is possible to differentiate the thrombus origin on pathologic examination but not clinically on ultrasound imaging. Wide variability exists for ultrasound diagnosis of EHIT and de novo DVT. Care must be taken in evaluating post-treatment duplex scans to not assign a diagnosis of EHIT when DVT may well be present and extending into the deep venous system. Whereas elimination of a thrombus may be the most efficient way of reducing collagen, the modulation of collagen production in the treatment of DVT may be helpful in preventing vascular dysfunction and reducing the post-thrombotic changes. Further studies on injury after RFA and laser ablation are needed and in larger numbers.

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REFERENCES


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