Detection of Thrombophlebitis with \(^{111}\text{In}\)-labeled Anti-Fibrin Antibody: Preliminary Results

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Abstract

Deep venous thrombosis remains a major medical problem, affecting a large segment of the population and resulting in significant mortality and morbidity.

Current techniques available for detecting deep venous thrombosis present limitations that may mitigate their potential benefit to the patient. Invasive techniques, such as ascending contrast venography, carry risks to the patient with regard to complications such as an allergic reaction to an iodine dye, adverse effects to renal function, and clot formation in a normal vein. Noninvasive techniques, such as Doppler ultrasound and impedance plethysmography, evaluate only a limited segment of the venous bed. The need remains for a diagnostic technique that is safe, accurate, and widely accessible.

A readily available noninvasive scintigraphic technique utilizing radiolabeled monoclonal anti-fibrin antibody may overcome some of these shortcomings. This imaging examination is quite effective in detecting clots in the lower extremities. Compared to contrast venography, \(^{111}\text{In}\)-labeled anti-fibrin antibody imaging appears to be as sensitive in identifying acute venous thrombosis. In addition, the preliminary data indicate that anticoagulation with heparin may interfere with adequate visualization of the clots with this technique.

Introduction

DVT remains a major medical problem, affecting an estimated 2.5 million patients/year and resulting in significant morbidity and mortality (1). Often insidious in that they are frequently asymptomatic, DVT and pulmonary embolism arising from DVT can elude clinical detection. Autopsy studies suggest that up to 84% of patients with pulmonary emboli were undiagnosed prior to death (2-4).

Current invasive and noninvasive diagnostic techniques are inadequate in visualizing thrombosis in the entire venous system in patients suspected of thromboembolism. In addition, invasive studies such as contrast venography carry considerable risks to the patient. Because of these shortcomings, the search for a technique that is safe, accurate, and widely accessible has continued for a decade.

In this communication, a new noninvasive scintigraphic technique, utilizing radiolabeled monoclonal anti-fibrin antibody, will be discussed for its potential application as an alternative diagnostic tool. After assessment of current diagnostic techniques for the diagnosis of deep venous thrombosis, monoclonal antibody immunoscintigraphy and its advantages over established diagnostic methods will be presented. Preliminary data obtained from the \(^{111}\text{In}\)-labeled anti-fibrin monoclonal antibody study, conducted at the Hospital of the University of Pennsylvania, will then be briefly discussed.

Assessment of Current Techniques

The method of choice in the clinical evaluation of deep venous thrombosis is ascending contrast venography. Considered the "gold standard" for clot detection, venography is capable of visualizing 95% of all clots (5, 6). Able to give a detailed view of the deep venous system, venography virtually assures detection of life-threatening clots in the extremity under study.

The limitations of venography are therefore not related so much to accuracy as to the materials used in the procedure, in which large quantities of contrast dye, typically between 100 and 150 ml, are injected into the patient (7). Patients with cardiac or renal dysfunction are not considered to be safe candidates for venography (6, 8). Patients may develop a reaction to the contrast agent which may contain as much as 20% iodine (9). Between 7.5 and 24% of venography patients experience discomfort including pain, swelling, tenderness, or erythema (7). One study found that 6.4% of patients had positive repeat venograms after an initial negative study (9).

In addition, because of the technical expertise required to perform the venogram, this study is not accessible to all patients. First, a suitable vein must be found; failure to cannulate a vein prevents a study from being conducted. Inadequate mixing of contrast materials with the unopacified blood, poor injection technique, muscle tensing, use of a tourniquet, and varicosities can confound interpretations of the study. Further, in order to avoid incomplete mixing of contrast medium and blood, large amounts of contrast medium should be used (10).

Since a large quantity of contrast medium can cause complications, it would appear that this by itself may introduce new problems.

In addition to contrast venography, IPG, the \(^{131}\text{I}\)-labeled fibrinogen study, Doppler ultrasound, real-time B-mode ultrasonography, and MRI are other techniques for detecting thrombi. These methods are noninvasive and are therefore of less discomfort and risk to the patient. While each method provides helpful information for the clinician regarding specific regions of the venous system, each, in turn, has its limitations which restrict its usefulness in detecting a wide spectrum of DVT.

IPG is a sensitive test for clots in the thigh. Through measuring changes in the electrical resistance or "impedance" proportional to changes in blood flow, thrombi may be located (11). One disadvantage of this method is its poor sensitivity to clots in the calf (12). False positive results which can be caused by muscle tension, arterial insufficiency, increased venous pressure secondary to congestive heart failure, or venoconstriction due to shock, account for between 7 and 22% of all studies (13).

Up to 21% of the studies are false negatives, possibly due to nonocclusive thrombi which do not completely block blood flow, collateral circulation, and skin stretching caused by the placement of a thigh cuff (13, 14).

Doppler ultrasound techniques are based on the Doppler "shift" or the change in the frequency of a light or sound wave...
when reflected from a moving source. A blood clot causes an
abnormal response due to decreased venous blood flow velocity
in the area of the clot (13). Like IPG, Doppler ultrasound
techniques are excellent in detecting thigh clots (popliteal,
femoral, and iliac veins); reported accuracies in Doppler ultra-
sound for this application are greater than 90% but have a
sensitivity of less than 50% in the region of the calf (13, 15). In
addition, as with IPG, nonocclusive clots can cause false nega-
tive results.

Unlike other noninvasive techniques, 125I fibrinogen is highly
sensitive (up to 92%) for clots in the region of the calf (16).
This method has its disadvantages as well: dependence on active
thrombus formation in order to detect the presence of a clot;
interference from surrounding structures such as bladder activ-
ity, which results in poor sensitivity to proximal venous throm-
busis; a waiting period of at least 24 to 48 h between injection
and detectable evidence of a clot; and results based on relative
counting rates from a nonimaging probe, not from γ-scintilla-
tion imaging.

Real-time B-mode ultrasonography produces a two-dimen-
sional picture of the structure being examined. A transducer
emits and detects sound waves in rapid succession, permitting
a “moving picture” to appear on the viewing screen. The time
of echo return and the direction in which the ultrasound beam
is reflected are used to construct the two-dimensional image.
Solid mass areas are characterized by more echoes (hyperech-
ogenicity) than fluid-filled areas. Studies using B-mode ultra-
sound to detect deep venous thrombosis have a sensitivity of
100% in the detection of the femoral and iliac thrombi; this
sensitivity drops to 62.5% for calf thrombi (17–19).

False positive results in real-time B-mode ultrasonography
will result when the patient has a hematoma, a popliteal cyst,
lower limb ischemia, cardiac or respiratory insufficiency, and
obesity (17–19). In addition, this method cannot conclusively
distinguish between acute and chronic thrombi. Given these
limitations, real-time B-mode ultrasonography is often used in
conjunction with other noninvasive studies such as IPG and
Doppler ultrasound.

MRI is an alternative noninvasive technique. MRI is based
on the observed phenomenon that a hydrogen atom will reso-
nate (produce a signal) at a frequency which is directly related
to the strength of the magnetic field to which it is exposed. The
strength of the signal will also depend on the properties of the
material (i.e., the density of hydrogen atoms in a given tissue).
The intensity of the energy emitted, where the timing and
strength of the magnetic field is known at any given point
within the space being scanned, can provide important anatom-
ical information.

The ability of MRI to detect DVT has been investigated; it
has been determined that MRI is an excellent technique from
which the resolution of a thrombi can be monitored; however,
slow blood flow or stasis may create problems in scan inter-
pretation (20). Thrombi can have magnetic properties that may
differ from other stationary tissue and from flowing blood. The
effects on signal intensity of slow blood flow in proximity to
the thrombus may not be measurably different (i.e., below a
threshold) from that of the thrombus itself, exaggerating its
size. Also, contrast quality is compromised when adjustments
are made to decrease this problem. Limited flip angle, gradient-
refocused MRI demonstrates some improvements over tradi-
tional spin-echo MRI; this method uses pulse sequences with
short repetition times and flip angles of less than 90 degrees
(21). The age of the thrombus may also contribute to the
variability in the MRI image obtained.

Every technique mentioned has its limitations which reduce
its effectiveness in the ability to unequivocally locate deep
venous thrombi. Several of the methods described are adequate
in the detection of DVT for specific regions of the body but are
inadequate (i.e., high false positive, false negative percentages)
for other regions. For example, impedance plethysmography
and Doppler ultrasound are adequate for examining the prox-
imal venous system but inadequate for the detection of thrombi
in the calves, while 125I-labeled fibrinogen is sensitive to clots
in the calves but not in the thighs. Contrast venography is
usually applied in examining only one extremity to reduce
contrast load and discomfort.

As an invasive technique, contrast venography carries with it
the risk of complications from the procedure including an
allergic reaction to the iodine-containing contrast medium,
while certain medical conditions, such as cardiac and renal
dysfunction, render participation in this procedure unsafe. In
Doppler ultrasound, nonocclusive thrombi can cause false nega-
tive results, whereas in MRI, the extent of the thrombus is
exaggerated by an area of slow blood flow resembling a clot.

Immunoscintigraphy: A New Noninvasive Technique

Considering the limitations of both invasive and noninvasive
techniques currently applied by clinicians for the detection of
DVT, it should be evident that a better imaging modality is
needed. Immunoscintigraphy, or the imaging of pathological
conditions using radiolabeled antibody and antibody fragments,
is a promising new technology that may help to address the
diagnostic inadequacies encountered in other techniques.

The production of monoclonal antibodies originated with
Kohler and Milstein (22) in 1975. A single hybrid cell, stimu-
lated to produce a specific antibody in response to a selected
antigen, is the parent cell for a colony of genetically identical
daughter cells. This colony or clone is referred to as a “hybrid-
oma”; the antibodies generated by the hybridoma are therefore
monoclonal. Monoclonal antibodies are biochemically identical
to one another, since they are produced by genetically identical
daughter cells from a single parent cell.

The selection of an antigen is a critical component in radioim-
munoimaging study. Fibrin, the biomolecule that forms the
structural matrix of the blood clot and the biochemical end
product in the process of blood coagulation, was chosen as the
antigen in this study. When clot formation is initiated, fibrin is
produced in relatively large quantities (mg amounts). It is
relatively accessible to the antibody, since the clot is produced
within the vascular space making it an ideal candidate as a
monoclonal antibody target. Platelets have been used as the
selected antigen in some studies, but it has been found that
monoclonal anti-platelet antibodies tend to aggregate at only
fresh thrombi sites (23–25).

Monoclonal anti-fibrin antibodies can be further processed
from whole antibodies into antibody fragments, which have the
advantage of being less likely to stimulate a harmful immune
response in the patient. This response is usually referred to as
producing human anti-murine antibody. Some studies have
been conducted using whole antibodies (26). In this study, a
Fab fragment (59D8) anti-fibrin antibody was used, in order to
avoid the potential problem of an allergic reaction developing
in the patient.

This antibody (59D8) is fibrin specific and does not react
with fibrinogen (the precursor of fibrin which is biochemically
almost identical and therefore potentially confusing to the
antibody). This antibody was developed by Hui et al. (27) and
was shown to detect clots for up to 4 days in rabbits and up to 24 h in dogs, in one study (28). Other studies confirm the efficacy of immunoscintigraphy to detect the presence of thrombi using the monoclonal anti-fibrin antibody T2G1 (29–31).

One potential advantage from using monoclonal antibody immunoscintigraphy over conventional techniques, such as IPG and Doppler ultrasound, is that it has the capability of detecting the presence of thrombi over the entire body, as opposed to a limited region. Another advantage is that, as a noninvasive procedure, it does not carry the risks associated with an invasive technique such as contrast venography. The following preliminary results indicate the potential usefulness of immunoscintigraphy in detecting DVT.

**Preliminary Results**

Eighteen patients were given injections of approximately 2.0 mCi of 59D8 anti-fibrin antibody Fab fragments labeled with 111In in conjunction with the chelating agent DPTA. The study group consisted of nine men and nine women ranging in age from 26 to 83 years. Eleven patients had undergone total knee or hip arthroplasty. Fifteen of these patients also underwent contrast venography; all but one of the venograms were performed within 48 h of antibody injection. One venogram was done approximately 96 h after the antibody study.

Most patients were imaged immediately after injection, 3 to 4 h postinjection, and 24 h postinjection. Two of those imaged immediately after injection had negative initial images, but positive images at 3 to 4, and 24 h postinjection. Otherwise, positive images were noted within 30 min after injection.

When compared with contrast venography, anti-fibrin antibody imaging appeared as sensitive in identifying patients with clots. However, there was some discrepancy in the latter technique in visualizing the abnormal sites. In the calf and popliteal regions of the leg, anti-fibrin matched all of the clots in venography, plus an additional site in each region. In the thigh, the venogram detected four thrombi, and the anti-fibrin study found only two. Both studies picked up the same single iliac clot.

Heparin had a confounding effect on antibody uptake. Although thrombi in these patients were still visualized and anti-fibrin scans actually picked up an additional site of clot in the popliteal and calf regions, uptake was decreased in the delayed images. This may be due to the fact that the presence of heparin prevents new thrombus formation and therefore allows the body's fibrinolytic activity to lyse the surface of the clot and detach the anti-fibrin-fibrin complex from the surface of the thrombus. Additional research on the effects of heparin therapy on anti-fibrin antibody uptake would be valuable in the evaluation of the efficacy of radioimmunoimaging in detecting DVT.

**Discussion**

The results suggest that radioimmunoimaging using 111In-labeled 59D8 Fab-DPTA monoclonal antibody is able to successfully detect thrombi in the regions of the lower extremities as confirmed by contrast venography. The images displayed significant uptake within 30 min of injection, suggesting that this technique can compete with other techniques in identifying patients with DVT for appropriate treatment. Heparin therapy decreases the sensitivity of the antibody and presents clinicians with the dilemma of when to begin heparin therapy, before or after definitive diagnosis of DVT.

Despite the existence of several methods for detecting DVT, the need remains for a safe, accurate, and widely available technique for diagnosing the condition. The invasive procedure of ascending contrast venography and the noninvasive techniques of impedance plethysmography, Doppler ultrasound, 125I-labeled fibrinogen, real-time B-mode ultrasound, and MRI lack one or more of the attributes described above. Our results indicate that the 111In-labeled 59D8 Fab-DPTA monoclonal antibody is accurate in targeting thrombi and that adoption of this technique would not require major investment in either the purchase of new equipment or the employment of highly skilled personnel. These studies can be adequately carried out using the existing facilities of modern nuclear medicine laboratories. Further investigation of radioimmunoimaging with monoclonal anti-fibrin antibodies may demonstrate that this method possesses similar or perhaps greater diagnostic capabilities without the liabilities of established procedures.

**References**

"In-Labeled Anti-Fibrin Antibody Detection of Thrombopyleitis"

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