Indium-111 platelet scintigraphy for the diagnosis of acute venous thrombosis

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ABSTRACT Platelets labeled with indium-111 have been used successfully as a marker of active thrombosis in man. To establish the diagnostic accuracy of platelet scintigraphy in comparison to contrast venography in the diagnosis of acute lower limb venous thrombosis, we evaluated 103 consecutive patients divided into two groups. Platelets were labeled by the indium-111 oxine method. Patients from group I (n = 73, 56 had venograms) were asymptomatic and underwent platelet scintigraphy 1.1 ± 0.6 days (mean ± 1SD) after a major orthopedic procedure. Patients from group II (n = 30, all had venograms) were symptomatic and underwent platelet scintigraphy 1.2 ± 1.7 days after venography. In group II, 15 patients with positive findings on contrast venography were treated with intravenous heparin; five others with positive venograms did not receive heparin until platelet scintigraphy was completed. Both platelet scintigraphy and contrast venography were evaluated by two blinded observers. Only studies with blinded agreement of both platelet scintigraphy and contrast venography were included in the analysis. Sensitivity and specificity of platelet scintigraphy for the whole limb were 93% and 97% in group I and 42% and 67% in group II. The lower sensitivity in group II was most likely attributable to therapy with heparin. These results demonstrate that platelet scintigraphy, a test that permits imaging for up to five days after a single injection, correlates favorably with contrast venography in patients who have not received heparin and may be used as a surveillance test in high-risk patients. The role of platelet scintigraphy in acutely symptomatic patients requires further evaluation.

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THE CLINICAL RECOGNITION of deep vein thrombosis is unreliable, lacking both sensitivity and specificity.1-3 In light of the potential hazards of anticoagulant therapy, an objective diagnosis of venous thrombosis is desirable before anticoagulant treatment is instituted. Currently, contrast venography is generally recognized as the reference diagnostic test. However, it may be uncomfortable for the acutely ill patient and is not suitable for repeated examinations. Complications are fortunately rare but include induced thrombosis and adverse effects of contrast material.4,5 The latter may be allergic, nephrotoxic, or hemodynamic. More importantly, even in the most experienced hands it is not always possible to visualize the entire venous system. Therefore efforts have been made to develop alternatives to venography. These include Doppler flow ultrasound,6,7 125I-fibrinogen leg scanning,8,9 and the various plethysmographic techniques,10 of which impedance plethysmography is the most commonly used. There is no consensus as to which of these techniques should be employed uniformly either singly or in combination.

The most thoroughly evaluated tests are impedance plethysmography and 125I-fibrinogen leg scanning. In combination they have been proposed as an alternative to venography.8,11 Fibrinogen scanning allows accurate recognition of thrombi in the calf, whereas impedance plethysmography is accurate above the knee. This approach, however, is not without difficulties. Fibrinogen scanning requires a delay of 24 hr before a defini-
tive diagnosis can be made. Because $^{125}$I is not readily imaged, interpretation of studies in the presence of hematoma or thrombosis of nonvenous origin may be difficult. Impedance plethysmography may be limited by false-negative studies that occur in patients with good venous collaterals and by false-positive studies that occur in patients in heart failure or in those with a nonthrombotic or nonacute proximal occlusion. Increased muscle tension may provide interpretive difficulties. Optimally performed by experienced investigators, the combination of fibrinogen scanning and impedance plethysmography carries a sensitivity of 94% and a specificity of 91% in symptomatic patients.

The role of indium-$^{111}$(I$^{111}$) platelet scintigraphy in identifying thrombosis in man has recently been reviewed. Both animal and human studies have demonstrated its potential value for the diagnosis of deep vein thrombosis. I$^{111}$ has physical characteristics suitable for imaging that facilitates interpretation of studies. Theoretically, thrombi may be identified in any location. The physical half-life of the isotope (2.8 days) and the biological life of the injected platelet (8 to 10 days) permit imaging for at least 5 days after injection of the platelet suspension. Thus monitoring of natural history and therapeutic response is possible, as is surveillance of high-risk patients.

The purpose of this study was to evaluate the accuracy of I$^{111}$In platelet scintigraphy in comparison with the recognized standard of contrast venography in both acutely symptomatic and high-risk postoperative patients.

Materials and methods

This study was approved by the Human Investigation Committees of the Universities of Yale and Oklahoma and of the West Haven and Oklahoma City Veterans Administration Medical Centers. Written informed consent was obtained from all patients.

Patients. A total of 103 patients, divided into two groups, was studied prospectively. Group I contained 73 men, aged 63 ± 12 years (mean ± SD), who were entered into the study before major lower limb orthopedic surgery, 45 after total hip replacement, 12 after total knee replacement, and 16 with limb fractures requiring alignment or pinning. Prophylactic therapy was determined by the primary physician and consisted of low-dose warfarin, (prothrombin time 1.5 × control, n = 7), aspirin (325 mg bid, n = 31), subcutaneous heparin (n = 6), and no therapy (n = 8). Before initiation of the study the primary physicians agreed that their usual prophylactic measures after surgery would be used. It was also decided that since platelet scintigraphy was an investigational technique with unknown accuracy, the results of these studies would not influence the management of these patients unless a venogram could not be performed after the platelet scan, in which case the primary physician would have access to the platelet scintiphotographs and could make an independent decision with regard to further management. All patients agreed to undergo both I$^{111}$In platelet scintigraphy and either unilateral or bilateral contrast venography after the operation. Patients were given the option of having unilateral or bilateral venograms. All unilateral venograms were performed on the operated side. The results of the platelet scans did not influence the decision to perform the venogram nor did they influence the side of the unilateral venography.

Labeled platelets were injected 1.1 ± 0.6 days (range 1 to 9) after surgery. Scintigraphy was performed within 4 hr of injection in 16 patients and within 48 hr in the remainder and thereafter on at least alternate days for a maximum of 5 to 7 days. In 21 patients venograms were not obtained because of patient refusal (n = 10), unstable renal function (n = 4), scheduling error (n = 2), and medical or surgical complications of surgery that either increased the risk of venography or presented major technical difficulties precluding the use of this technique (n = 5). Fifty-six venograms in 52 patients (four bilateral) were performed 6.8 ± 1.9 days (range 5 to 12) after surgery and 1.8 ± 1.7 (range 0 to 7 days) after the last platelet image, which was used for comparison with venography.

Group II patients (n = 30) entered into the study after a clinically motivated request for venography. Patients with positive venographic findings were treated with intravenous heparin unless contraindicated. A single set of platelet images was usually obtained after venography. The time from venography to injection of isotope was 1.2 ± 1.7 days (range 3 to 6 days). Images were obtained 13 ± 16 hr (range 1 to 48) after injection of the isotope.

I$^{111}$In platelet scintigraphy/venography. Platelets were separated from 43 ml of whole blood by centrifugation and labeled in acid citrate dextrose: saline (1:7) with I$^{111}$In 8-hydroxyquinoline at room temperature. After labeling, platelets were washed with plasma to remove loosely bound I$^{111}$In from the surface of the cell before injection back into the patient. A total of 414 ± 95 (mean ± 1 SD) μC I$^{111}$In was injected. Ten minute anterior images of the thigh, knee, and calf were acquired on either a wide-field-of-view or Technicare portable gamma scintillation camera. Both were fitted with a parallel-hole medium energy collimator with symmetric 20% windows set on the 173 and 247 keV photopeaks of I$^{111}$In. Typically 20,000 to 60,000 counts were obtained. Contrast venography was performed by the standard technique of Rabinov and Paulin. Films included calf and thigh.

Analysis of platelet scintigrams/venograms. Scintigrams of the whole limb were analyzed by two observers blinded to the result of the venogram. Each observer was provided the following information: location and type of operation, a scout film of the limb defining the site of the orthopedic prosthesis if present, surface hematology found on physical examination, and the location of prosthetic arterial grafts. This information was provided to closely simulate the clinical situation in which these images might be read in the future. Each observer was asked to grade the limb as positive, negative, or equivocal. Images were read in their temporal sequence, thereby allowing serial analysis of images for determination of regression or progression of existing thrombus. For the determination of diagnostic accuracy, only those single images most closely, temporally related to the venogram were used. The definition of a positive image was an area of increased activity corresponding to a vascular channel that extended beyond the direct area of surgery. Where doubt existed an equivocal reading was made. The analysis for sensitivity and specificity included only those platelet scans with total agreement by both blinded readers; 80 of 86 (93%) qualified for the final analysis.

Venograms were read by two blinded vascular radiologists who were unaware of the patient’s identity, the clinical history, or the results of platelet scintigraphy. Each observer was asked to grade the venograms as positive, negative, or equivocal. In
TABLE 1
Reproducibility of platelet scintigraphy and venography

<table>
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<tr>
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<th>Platelet scintigraphy</th>
<th>Venography</th>
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<tbody>
<tr>
<td>Interobserver agreement</td>
<td>80/86 (93%)</td>
<td>78/86 (91%)</td>
</tr>
<tr>
<td>Poor technical quality</td>
<td>0/86 (0%)</td>
<td>8/86 (9%)</td>
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*Includes studies with venograms.

Cases where the technical quality of the venograms precluded a diagnosis, the venogram was assigned to a technically inadequate group and excluded from the analysis. The criteria for acute thrombosis were a constant intraluminal filling defect with or without definite collaterals. Veins with an irregularly narrowed lumen with absence of normal valves were considered to have nonacute disease and these venograms were categorized as negative for acute thrombosis. For the analysis, as with the platelet scans, only those technically adequate venograms with blinded agreement of both observers were used; 70 of 86 (81%) qualified.

**Results**

**Venograms (table 1).** A total of 86 limbs from 82 patients were evaluated; the interobserver agreement for the whole limb was 78 of 86 (91%). Venograms of six limbs (7%) from group I and two limbs (7%) for group II were excluded from the analysis because of poor technical quality. Both observers agreed with the assessment of technical quality. In group I, 16 venograms were positive for thrombus, three proximal and 13 distal. In group II, 21 were positive; four proximal and seven distal alone and 10 with both proximal and distal thrombi.

**Accuracy of platelet scintigraphy per limb (table 2).** The interobserver agreement was 80 of 86 (93%). Technically inadequate venograms were excluded. The sensitivity and specificity for group I (n = 43) were 93% and 97%, respectively. In group II (n = 27) sensitivity was 42% and specificity 67%. The subset of the total population used in this final analysis, i.e., those limbs with technically adequate venograms and concordance between observers with the interpretation of both platelet scans and venograms, was 70 of 86 (81%).

The lower sensitivity in group II was most likely attributable to therapy with heparin, since four of five patients with positive venograms did not receive heparin and five of 15 who did had positive images (p < .13, Fisher exact test).

The effect of prophylactic therapy on the development of venous thrombi in group I was also evaluated. With technically adequate contrast venography as the method of evaluation, six of 31 receiving aspirin, four of seven on subcutaneous heparin, and none of five on warfarin alone developed thrombi. Of those patients not receiving prophylactic agents, three of eight developed thrombi. With platelet scintigraphy, rather than venography, as the method of evaluation, the only difference seen was in the aspirin group: seven of 31 by platelet scintigraphy compared with six of 31 by venography had thrombi.

In group I, only 16 limbs were imaged within 4 hr of injection of the platelet suspension. The diagnostic accuracy was 100%, with 14 true negatives and two true positives. In group I, six patients had progression (n = 1) or regression of thrombus (n = 5) (see figure 2). In none of the patients with regression of thrombus did the thrombus disappear completely.

**Preliminary analysis according to anatomic location (table 3).** In a separate analysis, each limb was divided into two segments above the knee and below the inguinal ligament (segment 1) and below the knee (segment 2). In other respects, the methods of analysis were identical to that already described. For venography the interobserver agreement was 153 of 172 (88%) for both segments from both groups. The total number of segments excluded from the analysis because of poor technical quality was 28 of 172 (16%), 16 from group I and 12 from group II. Thirteen were from segment 1 and 15 from segment 2. In group I, 16 segments were positive for thrombus, three in segment 1 and 13 in segment 2. In group II, 31 segments were positive with 14 from segment 1 and 17 from segment 2.

For platelet scintigraphy the interobserver agreement was 93% for all segments from groups I and II.
TABLE 3
Preliminary analysis according to anatomic location

<table>
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<tr>
<th></th>
<th>Group I venogram</th>
<th>Group II venogram</th>
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<tr>
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<td>+</td>
<td>-</td>
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<tr>
<td>Platelet scan</td>
<td></td>
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</tr>
<tr>
<td>Segment 1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Segment 2</td>
<td>1</td>
<td>41</td>
</tr>
<tr>
<td>Platelet scan</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>32</td>
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For group I–segment 1: sensitivity, specificity = 67%, 98%.
For group I–segment 2: sensitivity, specificity = 92%, 94%.
For group II–segment 1: sensitivity, specificity = 50%, 100%.
For group II–segment 2: sensitivity, specificity = 18%, 71%.

There was 3% disagreement between observers. For group I, sensitivity for segments 1 and 2 was 67% and 92%, respectively. The corresponding specificities were 98% and 94%. For group II the sensitivity for segments 1 and 2 was 50% and 18%, with specificities of 100% and 71%.

The high sensitivity in group I segment 2 and the correspondingly high specificity for both segments in group I points to the potential value of platelet scintigraphy as a means of diagnosing venous thrombosis irrespective of location along the limb. In group I only three patients had thrombi in segment 1. The sensitivity of 67% might therefore not represent the true accuracy of the technique. Larger numbers are required to resolve this question. In group II the lower-than-anticipated specificity (71%) in segment 2 is most probably related to venographically induced thrombosis. The low sensitivity in both segments in group II (50% and 18%), as was seen in the analysis for the whole limb, is most likely attributable to the effect of heparin. If this is the case, we speculate that the effect of heparin is more pronounced in distal rather than proximal thrombi.

Discussion

Since Thakur et al. first reported success in labeling platelets with [111In]8-hydroxyquinoline in 1976, there have been several studies demonstrating the value of platelet scintigraphy as an investigative tool for monitoring platelet behavior in vivo. However, it is for the identification of mural left ventricular thrombi16, 28-33 and deep vein thrombosis16, 17, 20-23 that platelet scintigraphy has shown most promise for possible use in the clinical arena. In the case of venous thrombosis, it may obviate some of the disadvantages of existing methods and may offer advantages not available with other techniques. In the largest reported series before this report, Fenech et al.23 successfully imaged 24 of 26 venographically detected thrombi in a population of both high-risk surgical patients and those with clinically suspected disease.

Advantages. The primary advantage of this technique is that it is a noninvasive test that may be brought to the bedside by means of a portable camera for patients in intensive care units. Furthermore, high-quality platelet images may allow accurate localization of thrombi to a particular area irrespective of site along the limb.22, 23 The pattern of uptake attributable to venous thrombi is characteristic (figures 1 and 2) and can be distinguished from other entities associated with the uptake of platelets. The latter include wounds, subcutaneous hematoma, trauma from implantation of orthopedic prostheses, platelet uptake on vascular grafts, and localized areas of infection. Scintigraphy is possible for between 5 and 7 days after injection of the labeled platelet suspension. Thus surveillance of high-risk patients and the monitoring of extension and regression is possible, although the clinical relevance of the latter needs to be defined.

The effect of heparin on scintigraphy may be both advantageous and disadvantageous. Most patients with positive thrombi by venography received intravenous heparin (group II), and the sensitivity for detecting thrombi with platelet scintigraphy decreased from 93% in group I to 47%. This reduction in sensitivity was most probably attributable to heparin, since five of the five patients with positive venograms had positive platelet scintigrams. Thus future studies may be directed at monitoring the efficacy of anticoagulant therapy. The effect of heparin as evidenced by platelet scintigraphy has been observed by other investigators.17-19 Conflicting evidence failing to show this effect has also been reported.22, 23 We believe this does represent a true phenomenon and is not due to the effect of contrast agents, which have been shown not to affect the accuracy of platelet scintigraphy in detecting pulmonary embolism in dogs.19 Thus, on the one hand, platelet scintigraphy may be of potential value for directly monitoring efficacy of therapy; however, adequate therapy with heparin may reduce the sensitivity of the technique when used for diagnosis.

Although images of the chest were not obtained routinely, an area of increased [111In activity in the lungs corresponding to an angiographically documented pulmonary embolus was seen in one patient. Thus this technique, as has been suggested previously, is poten-
FIGURE 1. $^{111}$In scintigraphic image shown in the right panel is a 10 min image containing 39,000 counts. Platelets were injected 48 hr after total hip replacement with the image acquired 24 hr later. The corresponding venogram is adjacent. F = focal uptake; R = right leg; L = left leg; C = filling defects caused by venous thrombosis.

This application, however, requires evaluation.

**Disadvantages.** There are two important disadvantages of this technique. First, platelets require labeling in vitro in a sterile environment necessitating 90 min of skilled technician time. Second, the practice of placing patients on heparin before obtaining an objective diagnosis will in most cases require the discontinuance of therapy before imaging. The development of labeling techniques in vivo using antibodies to platelet recep-

FIGURE 2. Two images from the same patient obtained 3 and 72 hr after injection of the platelet suspension. Panel B represents progression of the thrombus over the 69 hr between images.
tors promises to overcome the need for labeling in vitro. This new approach, if shown to be as efficacious as the in vitro methods, would greatly enhance the general availability of platelet imaging.

Experience with interpretation of studies, particularly with respect to the recognition of conditions that might produce false-positive images, is essential to ensure quality control and accurate diagnosis. Venous thrombi in the region of the surgical site may be difficult to identify if platelets are injected before or immediately after surgery. With normal wound healing these difficulties may be obviated by the injection of the platelet suspension 24 to 48 hr after surgery. Our results in the postoperative orthopedic group indicate that potential difficulties related to surgical sites and the problems of imaging immobilized patients, often with casts, can be overcome.

Radiation exposure. The literature concerning dosimetry calculations has been summarized by Powers and Siegel. In this study, 400 ± 95 μCi 111In was used, 500 μCi being the maximum allowable dose permitted by our IND. Approximately 20% to 30% of the radio-labeled platelets localize promptly to the spleen, leading to an estimated exposure of 25 to 34 rems/mCi. The radiation exposure per millicurie to other tissues is considerably less: 0.6 to 4.2 rems to the liver, 0.5 to 1 rems to the bone marrow, 0.1 to 0.8 rems to the gonads, and 0.3 to 0.9 rems to the whole body.

Future directions. This study demonstrates that platelet scintigraphy may be used as a surveillance tool for deep vein thrombosis in high-risk elderly male patients. Yet to be shown is the value of this technique in the diagnosis of deep vein thrombosis in those patients clinically suspected of having the disease. For platelet scintigraphy to compete favorably with currently available techniques, it must be shown to be accurate along the whole limb (an added major benefit would include pelvic vein thrombosis) and to provide accurate information within 4 to 5 hr after injection of the isotope. This may be possible because the problem of high background encountered with imaging coronary thrombi is greatly reduced in the peripheral circulation. In addition, the influence of commonly encountered antiplatelet and anticoagulant drugs must be defined. A study based on information gained from this study is currently underway to address these issues. If these initial prerequisites are met, important added advantages of platelet scintigraphy, such as quantification of images, thereby allowing the direct monitoring of therapeutic efficacy, will come into play. It would be important also to compare this technique with currently available techniques other than venography, including impedance plethysmography and Doppler ultrasound. Unfortunately, a direct comparison between platelet scintigraphy and fibrinogen scanning may not be possible because of the inability to distinguish simultaneously, between the two isotopes currently used for labeling these agents.

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