Platelet Deposition on Dacron Aortic Bifurcation Grafts in Man: Quantitation with Indium-111 Platelet Imaging

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SUMMARY The interaction between platelets and chronically implanted prosthetic arterial graft surfaces in man was assessed by imaging indium-111-labeled platelet deposition in 15 patients with Dacron aortic bifurcation grafts. Grafts had been in place for 9–120 months. These results were compared with those obtained by platelet imaging of the aortofemoral vessels of 13 normal young adults without grafts. Quantitative evaluation was performed in both groups by a graft/blood ratio that compared indium-111 platelet activity in the aortofemoral vascular region to whole blood platelet activity over a 96-hour imaging time. In addition, blinded qualitative visual interpretation was performed on all studies by comparing aortofemoral area activity with that in adjacent native arteries. The reproducibility of the technique was evaluated by repeat imaging and quantitative analysis of six patients with grafts.

Quantitative evaluation of the normal subjects revealed that the mean vessel/whole blood activity ratio was 2.0 ± 0.7 (± SD) at 24 hours after platelet injection and remained constant for the duration of the study (1.7 ± 0.9 at 96 hours, NS). In contrast, patients with grafts had a mean graft/blood ratio of 3.0 ± 1.6 at 24 hours, which increased progressively to 7.8 ± 5.0 at 96 hours (p < 0.001 vs normal subjects), documenting ongoing platelet deposition in patients with vascular grafts. Independent qualitative visual interpretation of the aortofemoral region disclosed no abnormal uptake in normal subjects, whereas 13 of 15 patients (87%) with grafts had platelet deposition. The six reproducibility studies in patients with grafts were quantitative-ly unchanged and remained visually positive.

Platelet deposition in chronically implanted Dacron arterial grafts was consistently present, progressive over 96 hours, quantifiable and reproducible. These results imply absent or incomplete endothelial cell coverage of the graft flow surface. The techniques described may be useful in assessing prosthetic material thrombogenicity and the efficacy of platelet-inhibitory drugs in man.

THROMBOSIS and thromboembolism are well recognized complications of intravascular prostheses. Inadequate measures for assessing prosthetic thrombogenicity in man have impeded the development of improved materials. The finding that selective platelet consumption, without associated fibrinogen consumption, occurs in patients with recently implanted Dacron aortofemoral grafts, arteriovenous shunts and prosthetic cardiac valves is indirect evidence that platelets have a predominant role in the thrombotic response to arterial prostheses. This conclusion is also supported by the finding of platelet deposition on recently implanted prosthetic grafts in baboons, rats and dogs. However, the detection of platelet deposition on chronically implanted grafts in animals or man has been uncommon.

Coverage of the graft flow surface with endothelial cells in experimental animals decreases both platelet deposition and the thrombotic and embolic complications of arterial prostheses. Progressive and complete endothelial cell coverage of short Dacron grafts occurs rapidly after implantation. In baboons, the progressive increase in endothelial coverage was associated with normalization of platelet survival over a 12-week period. However, the cellular changes occurring on the graft luminal surface in humans have not been fully characterized. Normalization of shortened platelet survival times within 9 months after graft implantation in humans has been used as indirect evidence of endothelialization of the graft surface. However, histologic studies have rarely documented the presence of an endothelial surface in man. Thus, the available human data do not clarify the relative roles of platelets, endothelium and other surface alterations in the changing thrombotic response to Dacron arterial prostheses.

To study the interaction between platelets and Dacron vascular grafts in man, indium-111 platelet labeling and quantitative analysis of platelet uptake were performed (1) to determine the circulating platelet activity present in the aortofemoral area in healthy subjects without prosthetic grafts; (2) to define the frequency with which patients with chronically implanted (9 months or longer) Dacron aortic bifurcation grafts have externally detectable platelet deposition in the graft; (3) to assess the reproducibility of platelet imaging results; and (4) to assess quantitatively the dynamics of platelet deposition in Dacron arterial grafts over a 96-hour imaging time.

Methods

Subjects

Two groups were studied. One group consisted of 13 healthy volunteers, mean age 26 years (range 18–37 years). No normal subject received any medication known to affect platelet activity within 1 week before or during the study (aspirin, aspirin-containing compounds, dipyridamole, heparin, warfarin, or nonsteroidal antiinflammatory agents, including sulfinpyra-
zone). The second group consisted of 15 patients, ages 54–71 years, in whom aortofemoral or aortolilac prosthetic grafts had been in place 9–120 months (mean 26 months). All patients in this group had grafts constructed of knitted Dacron. Six patients had DeBakey-type grafts, eight had Sauvage-type grafts and one patient had an unknown type of knitted Dacron graft in place for 10 years. The duration of graft placement was not significantly different between patients with DeBakey (15 ± 2 months) and Sauvage (22 ± 7 months) grafts (NS). The major difference between DeBakey and Sauvage grafts is the external surface construction, which is smooth in DeBakey grafts, but relatively irregular velour in Sauvage grafts. Eleven patients with grafts had not taken any medications known to affect platelet behavior within 1 week before or during the study. One patient was on long-term ibuprofen (400 mg three times daily) and three used various combinations of aspirin plus dipyridamole (aspirin, 325 mg twice daily plus dipyridamole, 25 mg four times daily; aspirin, 650 mg plus dipyridamole, 100 mg every day; and aspirin, 325 mg plus dipyridamole, 75 mg three times daily). In these patients, platelet-active medications had been administered for at least 1 month before study and were continued throughout the study. Graft occlusion or embolization to the lower extremities had not occurred in any patient.

The normal volunteers had only one study. Six patients with grafts had repeat studies at a mean of 9.5 ± 6.1 weeks (range 2–16 weeks) to assess the reproducibility of the quantitative and visual platelet imaging findings. No patient had a change in medication or clinical status in the interval between studies.

This study was approved by the University of Washington Human Subjects Review Committee. All subjects gave informed consent.

Platelet Labeling and Imaging

Autologous indium-111 platelet labeling was performed using a closed bag modification of the technique of Thakur et al.,21 as previously described.22 For all 34 studies performed, the mean labeling efficiency was 30 ± 8% (± SD). The mean dose injected was 274 ± 17 μCi in the 13 studies of normal subjects, 347 ± 87 μCi in the 15 baseline graft studies, and 355 ± 95 μCi in the six reproducibility studies. For all 34 studies, the mean percentage of indium-111 present in platelet-poor plasma was 5 ± 2% at 24 hours, 6 ± 2% at 48 hours, 7 ± 3% at 72 hours and 8 ± 4% at 96 hours after injection.

Imaging was performed using a Sigma 410 gamma scintillation camera (Ohio Nuclear) and a medium-energy, parallel-hole collimator (model 28W08610). Both gamma photon peaks of indium-111 (173 and 247 keV) were collected using a 15% energy window. All images were recorded on both trilens Polaroid film for visual assessment of the unprocessed images and on a computer disc system with a 128 × 128 matrix to allow quantitation of the image data. Anterior views of the lower abdomen, which largely excluded the liver and spleen, were obtained for 150,000 counts. The imaging time was recorded. All normal subjects had images obtained at 24, 48, 72 and 96 hours after labeled platelet injection. Some patients with grafts could not return for multiple images. In the 15 baseline graft studies, imaging was discontinued at 48 hours in one patient, 72 hours in five patients and 96 hours in nine patients. In the six graft reproducibility studies, imaging was discontinued at 48 hours in one patient, 72 hours in three patients and 96 hours in two patients.

Data Analysis

Visual Analysis

Unprocessed images from the normal subjects and from the patients with prosthetic grafts were interpreted independently by two observers who were blinded to all clinical information except the imaging time. Images were defined as positive for abnormal platelet deposition if there was an area of activity present in the aortofemoral region that was clearly greater than the large-vessel blood pool activity present above or below the area encompassed by an aortofemoral graft. Studies were graded as positive, negative or equivocal for abnormal platelet deposition. For positive or equivocal studies, we noted whether the pattern of platelet deposition was diffuse or multifocal and whether there was any visual change in the pattern on serial imaging. Reproducibility studies were read with the baseline studies and were similarly graded as positive, negative or equivocal. We also noted whether there was any difference in the pattern of platelet deposition between baseline and reproducibility studies. Overall, there was observer agreement in 33 of 34 studies as to a positive, negative or equivocal reading; in one study, a consensus was reached.

Quantitative Image Analysis (Graft/Blood Ratio)

A quantitative index comparing gamma camera–derived indium-111 platelet activity in the aortofemoral graft region to platelet activity in well-counted whole blood samples was obtained for each patient at each imaging time. This index was expressed as a ratio of graft activity to whole blood activity. For each subject, the gamma camera–derived aortofemoral graft activity was determined by a hand-drawn region of interest encompassing the entire extent of an aortofemoral graft, or similar aortofemoral blood pool area in normal subjects (fig. 1). Activity from an adjacent background region was also determined from a region of interest just lateral to the left limb of the graft. All images on a given patient were shifted using a computer program so that the aortofemoral blood pool and anatomic markers on each image were located at the same position in a 128 × 128 computer matrix. The anatomic radioactive markers were placed 8.0 cm to each side of the umbilicus on each image to aid in the computer shifting. For each subject, the same graft and background regions were sequentially applied to the 24, 48, 72 and 96-hour images to ascertain serial total graft counts and background counts, which were normalized to a 1000-second imaging time. The total aortofemoral graft counts at each imaging time were cor-
rected for background nongraft activity by subtracting the average number of counts per channel in the background region from each channel in the aortofemoral region. The background correction was performed to minimize the contribution of count activity from adjacent small vessels and underlying bone marrow, which is a site of sequestration of approximately 4% of injected platelets.21

The graft/blood ratio was calculated at each imaging time by dividing background-corrected aortofemoral graft counts by indium-111 counts present in 0.1 ml of whole blood. Whole blood counts were obtained from a 5-ml sample drawn at the time of each image and counted for 200 seconds in a well counter. One normal subject did not have serial whole blood samples and was thus excluded from the quantitative analysis. In subjects without ongoing platelet deposition in the vessel or graft, both the aortofemoral graft region counts and the whole blood counts measure platelet counts in circulating blood. Since platelet senescence and isotope decay affect both measures equally, the graft/blood ratio should not change over time. In contrast, subjects with increasing deposition in the graft region over time should manifest increasing graft/blood ratios.

Intraobserver reproducibility for determination of the graft/blood ratio was assessed by blinded, repetitive analysis of 12 patient studies separated by at least 2 weeks. Interobserver reproducibility was assessed by having two investigators perform independent, blinded analysis in 10 patient studies. For intraobserver reproducibility, the correlation coefficient between the first and second analysis was \( r = 0.90 \) and for interobserver reproducibility, the correlation coefficient between two observers was also \( r = 0.90 \). Thus, the methods involved in calculating the graft/blood ratio were considered reproducible.

Statistical Analysis
Statistical analysis was performed using \( t \) tests for paired and unpaired data. Values are expressed as the mean \( \pm \) SD.

Results
Visual Analysis — Baseline Studies
By visual analysis, none of the 13 normal subjects had abnormal platelet deposition. Serial images of a normal subject are presented in figure 2. In early images, blood pool activity was clearly seen in the large vessels of the abdomen. Over the 96-hour imaging period, blood pool activity decreased progressively as senescent platelets were removed from the circulation. No focal areas of activity suggestive of abnormal platelet deposition in a vessel wall were observed in any of the normal subjects at any imaging time.

In contrast, 12 of 15 baseline studies in patients with...
prosthetic grafts were visually positive for abnormal platelet deposition, one study was equivocal, and two studies were negative. One patient with a Sauvage graft in place for 2 years had an equivocal study. One patient with a DeBakey graft in place for 1 year and one patient with a Sauvage graft in place for 2 years had visually negative studies. Visually, the Sauvage and DeBakey grafts were indistinguishable in their patterns of platelet deposition. Positive studies were seen in grafts of all ages, including one graft that had been in place for 10 years.

In the 12 positive and one equivocal baseline studies, the pattern of deposition for the entire set of images was graded as either diffuse and regular throughout the extent of the graft or as multifocal and irregular. Four patients had diffuse platelet deposition, which did not change over the imaging time (fig. 3). Nine patients had an irregular pattern of deposition. In five of these nine, diffuse deposition was present as well, while the four other patients had abnormal deposition visually detectable in only portions of the graft. In six of the patients with irregular deposition, the pattern of abnormal platelet uptake changed over time. Most typically, new areas of deposition appeared at later imaging times. In a few patients, areas of abnormal platelet deposition decreased or disappeared on serial imaging, compatible with disaggregation or downstream embolization of labeled platelet thrombus.

Quantitative Analysis — Baseline Studies

The graft/blood ratio in normal subjects remained constant from 24 hours (2.0 ± 0.7) to 48, 72 and 96 hours (1.8 ± 0.6, 1.7 ± 0.8 and 1.7 ± 0.9, respectively; all NS vs 24 hours), reflecting equal loss of indium-111 platelet activity from both the background-corrected vascular region of interest and from the well-counted whole blood (table 1, fig. 4). In contrast, among the 15 baseline graft studies, the graft/blood ratio at 24 hours was 1.5 times higher than that in normal subjects (3.0 ± 1.6; p = 0.07 vs normals), and increased progressively at 48, 72 and 96 hours (4.7 ± 2.5, 5.6 ± 3.6 and 7.8 ± 5.0, respectively; all p < 0.001 vs normals). In patients with grafts, the mean 48-, 72- and 96-hour graft/blood ratios were significantly increased from the 24-hour value (all p < 0.01).

Visual and Quantitative Analysis — Reproducibility Graft Studies

In all six patients with reproducibility studies, the repeat study was visually graded the same as the baseline study, which was positive in five patients and equivocal in one patient. Three patients with diffuse deposition on the baseline study continued to have diffuse deposition on repeat study and three patients with multifocal deposition initially also had multifocal deposition on repeat study. The distribution of multifocal deposition varied slightly between the initial and repeat studies, however. The graft/blood ratio of the reproducibility studies was not significantly different from the initial baseline studies at any imaging time (table 1).

Relationship of Quantitative and Visual Analysis

Two baseline and reproducibility graft studies were visually negative and two were equivocal. The graft/blood ratios of the visually negative or equivocal graft studies were increased compared with the normal subject studies, but were less than the visually positive graft studies (table 1). Moreover, the graft/blood ratio in these visually negative or equivocal studies increased from 24 hours to 48, 72 and 96 hours, unlike

<table>
<thead>
<tr>
<th>TABLE 1.</th>
<th>Background-corrected Graft/Blood Ratios</th>
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<tr>
<td></td>
<td>24 hours</td>
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<tr>
<td>Normal subjects (n = 12)</td>
<td>2.0 ± 0.7</td>
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<tr>
<td>All baseline graft studies (n = 15)</td>
<td>3.0 ± 1.6</td>
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<tr>
<td>Reproducibility studies</td>
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<tr>
<td>Baseline 1 (n = 6)</td>
<td>3.3 ± 1.8</td>
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<tr>
<td>Baseline 2 (n = 6)</td>
<td>3.1 ± 1.8</td>
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<tr>
<td>All visually positive graft studies (n = 17)</td>
<td>3.4 ± 1.5*</td>
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<tr>
<td>All visually equivocal or negative graft studies (n = 4)</td>
<td>1.4 ± 0.8</td>
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<tr>
<td>Graft studies in patients on platelet-active drugs (n = 6)</td>
<td>3.3 ± 1.6*</td>
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<tr>
<td>Graft studies in patients not on platelet-active drugs (n = 15)</td>
<td>2.9 ± 1.6</td>
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<tr>
<td>All DeBakey graft studies (n = 10)</td>
<td>2.9 ± 1.6</td>
</tr>
<tr>
<td>All Sauvage graft studies (n = 10)</td>
<td>3.3 ± 1.6*</td>
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Values are mean ± sp.
*p < 0.05 vs normal subjects.
the studies in normal subjects. Thus, over the 96-hour imaging time, indium-111 platelet activity in the graft region serially and significantly increased relative to whole blood activity in all graft studies, indicating ongoing platelet accumulation in the graft. The change in graft/blood ratio over time improved discrimination between normal subjects and patients with grafts that did not visualize.

The four patients who were receiving platelet-active medications had a total of six baseline and reproducibility studies, all of which were visually positive. The mean graft/blood ratio of these six studies was not significantly different at any imaging time from the 15 studies in patients who were not receiving platelet-active medications (table 1). Patients with Sauvage and DeBakey grafts did not differ significantly in graft/blood ratios at any imaging time (table 1).

In one patient, a 1-cm² section of Dacron graft was obtained 24 hours after platelet labeling for histologic study. (This patient is not included in the above results because no imaging was performed.) By transmission electron microscopy, a compact, thin layer of both fibrin and platelets was found on the flow surface. No endothelial cell coverage was present. The indium-111 activity of the full thickness of the graft compared to whole blood was 2.4:1 (corrected for weight).

**Discussion**

Platelet imaging provides an in vivo, organ-specific, noninvasive and quantitative index of abnormal deposition. This contrasts with prior tests of platelet function, such as aggregation, adhesion, platelet-specific protein release or platelet survival, which are either in vitro or do not anatomically localize the site of abnormal platelet activation. Indium-III platelet imaging has demonstrated platelet deposition in a variety of vascular diseases in man, including left ventricular thrombi, dysfunctional abdominal aortic aneurysms, prosthetic arterial grafts, arteriovenous fistulae, deep venous thrombosis, and carotid atherosclerosis. This study documents that platelet deposition commonly occurs in chronically implanted Dacron arterial grafts in man and quantitates the magnitude of this response. By visual analysis, 12 of 15 such grafts had definite indium-111 platelet deposition. By quantitative analysis, all grafts were abnormal, including the visually negative and equivocal studies, which had modestly elevated graft/blood ratios compared with those in normal subjects as well as an increase in this ratio over time. These quantitative findings suggest that platelet deposition occurred in the visually negative and equivocal grafts, albeit more slowly than in the visually positive studies.

Our visual and quantitative results suggest that platelet deposition in Dacron grafts was a dynamic and changing process over the 96-hour imaging time. Overall, most studies appeared visually more positive at later (72- or 96-hour) imaging times. The increase in graft/blood ratios over time more clearly indicates that progressive platelet deposition occurred in prosthetic grafts. This conclusion is in accord with the preliminary report of Heyns et al., which showed a progressive net increase in indium-111 platelet activity in abdominal aortic aneurysm thrombi over a 1-week period. Ultimately, a steady state in which the platelet deposition rate equals the dissolution rate must develop, or graft occlusion would occur. In the current study, the increasing graft/blood ratio indicates that such an equilibrium between deposition and removal...
of labeled platelets or labeled platelet products was not reached during a 96-hour study period.

Our method of image quantitation was designed to allow comparison of different patient groups or different studies in a given patient. Since the injected dose, platelet survival and platelet recovery change from study to study, an index that was independent of these variables was needed. Division of the gamma-camera-derived graft region activity by well-counted whole blood activity accomplishes this goal, because differences between studies in any of these variables equally affects both the numerator (background-corrected graft counts) and the denominator (simultaneous whole blood activity) of the graft/blood ratio. Similarly, over the 96-hour imaging time in a given study, isotope decay affects both the graft region and whole blood activities identically. Hence, the graft/blood ratio normalizes for interpatient and temporal differences in graft activity due to injected dose, platelet survival, platelet recovery and isotope decay. This index is also easily obtained by conventional gamma camera imaging and does not require whole body counting or imaging. The graft/blood ratio does not correct for variations in isotope attenuation among patients, which are largely related to body size. In the current study, there were no significant differences in body surface area between control subjects (1.96 ± 0.19 m²) and patients with grafts (1.89 ± 0.24 m²). A background correction was used because the region of interest for determining graft activity contained some counts from tissues surrounding the graft. One source of these non-graft counts is the pelvic bone marrow, which sequesters senescent platelets and may spuriously increase counts in the graft region. Therefore, the background region was drawn to encompass a portion of the pelvic bone marrow.

In the absence of abnormal platelet accumulation, the graft/blood ratio in effect represents two different approaches to measuring circulating intravascular labeled platelet activity in the aortofemoral region; thus, one would predict no change over time, as was observed in normal subjects. The progressive increase in this ratio over time in graft patients largely reflected increases in graft region activity per se, since both the whole blood and background activities changed similarly over time in patients with grafts and normal subjects.

Overall, the graft/blood ratio, which normalized for most interpatient variability and was shown to be reproducible, better discriminated between graft patients and normal subjects than did visual analysis alone. Moreover, the dynamics of platelet deposition over time were defined. This quantitative measure of platelet deposition is applicable to other sites and mechanisms of thrombus formation. Additionally, since clinical trials of platelet-active drugs to date have not resolved the issues of which antithrombotic agents and dosages are optimal, direct in vivo quantitative assessment of drug efficacy using this technique may be helpful.

The demonstration in this study of platelet deposition in chronically implanted human grafts differs from prior histologic studies, which have rarely reported significant platelet deposition. However, fixation techniques required to preserve platelet morphology may have been inadequate. In the current study, histologic examination by electron microscopy of a single specimen revealed platelets in large numbers on the luminal surface but no endothelial cells. This observation, and the increasing indium-111 graft activity over time, support our thesis that platelets deposit and accumulate progressively on graft surfaces over a 96-hour period. Continued deposition implies incomplete endothelialization of the graft flow surface and is compatible with histologic studies of grafts removed from humans, which have rarely documented endothelial coverage. An alternative explanation for continued platelet deposition would be the formation of a morphologically normal, but functionally abnormal, endothelium, as suggested in one animal study.

Endothelial cell coverage is theoretically desirable because it should decrease thrombotic and embolic complications. Thrombotic occlusion limits the use of prosthetic grafts to large, high-flow arteries. However, even large-caliber aortofemoral prostheses have a 10-26% incidence of thrombosis in one of the graft limbs by 5 years. Thrombotic occlusion is more frequent in smaller-caliber prosthetic grafts, such as femoropopliteal bypasses, in which 36-54% undergo occlusion by 1 year, and 50-62% by 5 years. Clinically detected embolic complications of aortic grafts in man are rare, however, distal embolization from prosthetic graft surfaces has been documented to occur frequently in animal models.

The finding of a thrombogenic surface on chronically implanted Dacron grafts in man differs from animal studies, in which endothelial cell coverage of the inner graft flow surface regularly occurs. Progressive endothelial coverage is associated with normalization of platelet survival over a 6-week period in baboons. Although platelet survival times normalize in some patients after Dacron arterial grafting, the current study suggests that the prolongation of platelet survival does not reflect graft endothelialization.

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