Technetium 99m–Labeled Annexin V Scintigraphy of Platelet Activation in Vegetations of Experimental Endocarditis

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Background—The pathophysiology of infective endocarditis involves a pathogen/host tissue interaction, leading to formation of infected thrombotic vegetations. Annexin V is a ligand of phosphatidylserines exposed by activated platelets and apoptotic cells. Because vegetations are platelet-fibrin clots in which platelet proaggregant activity is enhanced by bacterial colonization, we investigated the ability of annexin V labeled with technetium Tc 99m ($^{99m}$Tc-ANX) to provide functional imaging of these vegetations in experimental models of infective endocarditis. This ability was assessed in rabbits and rats because of the different interest of these 2 species in preclinical analysis.

Methods and Results—Nonbacterial thrombotic endocarditis was induced with the use of a catheter left indwelling through the aortic or tricuspid valve, and animals were injected with either a bacterial inoculum or saline. Scintigraphic investigations were performed 5 days later and showed a higher $^{99m}$Tc-ANX uptake by vegetations in infected versus noninfected animals (ratio, 1.3 for in vivo acquisitions and 2 for autoradiography; $P<0.0001$ for all), whereas no significant uptake was present in controls. Right-sided endocarditis was associated with pulmonary uptake foci corresponding to emboli. Histological analysis of vegetations showed a specific uptake of $^{99m}$Tc-ANX at the interface between circulating blood and vegetation. In parallel, underlying myocardial tissue showed myocyte apoptosis and mucoid degeneration, without extracellular matrix degradation at this stage.

Conclusions—$^{99m}$Tc-ANX is suitable for functional imaging of platelet-fibrin vegetations in endocarditis, as well as embolic events. $^{99m}$Tc-ANX uptake reflects mainly platelet activation in the luminal layer of vegetations. This uptake is enhanced by bacterial colonization. (Circulation. 2008;117:781-789.)

Key Words: endocarditis ■ imaging ■ nuclear medicine ■ thrombus ■ valves

Infective endocarditis remains a diagnostic and therapeutic challenge, as evidenced by the stability of its incidence over time and its morbidity and mortality rates.1,2 In addition to the direct valvular damage due to endocardial vegetations, embolic events are frequent and life-threatening complications of infective endocarditis.3 In this context, morphological imaging such as echocardiography is useful for the diagnosis and may have a prognostic value in predicting embolic events.4

Clinical Perspective p 789

The endocardial thrombotic vegetation represents a specific model of pathogen/host tissue interaction, involving the formation of a septic thrombus leading to injury of both underlying valvular and cardiac tissue and to possible peripheral septic dissemination.5 Pathogen-platelet molecular interactions are probably one of the main determinants of vegetation formation6 and growth that are linked to septic thrombus formation, including platelet activation and aggregation7 and fibrin-fibronectin deposition.8,9

The pathological consequences of vegetation formation, including degradation of the septic thrombus itself, leading to peripheral emboli,10 and of the underlying valvular and myocardial tissue, are probably linked to interactions between proteases of microbial origin11 and proteases conveyed by the thrombus.12 These proteases induce procoagulant and fibrinolytic activities10 within the vegetations and induce neighboring tissue injury.

Annexin V specifically binds with nanomolar affinity to phosphatidylserine, which is exposed on the surface of

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activated platelets and apoptotic cells. Therefore, annexin V radiolabeled with technetium Tc 99m (99mTc-ANX) has been used previously for in vivo imaging of both apoptotic cells in animals and humans and acute platelet-rich thrombi in animals. In a recent study, we have shown the ability of 99mTc-ANX to assess the luminal renewal activity of chronic aseptic mural thrombi in an experimental model of abdominal aortic aneurysm.

Because endocardial vegetations and secondary embolic events can be regarded as septic platelet-rich thrombi, we investigated the ability of 99mTc-ANX to provide functional imaging of endocardial vegetations and their embolic consequences using the classic experimental model of induced infective endocarditis in animals. This ability has been assessed in both left- and right-sided vegetations and in 2 species: the rabbit model, which is the canonical one, and the rat model, which is more suited to small-animal imaging devices, to immunohistochemistry analysis, and for further experimental therapeutic studies.

### Methods

**Experimental Models**

A summary of experimental models is presented in the Table. Aortic valve thrombotic vegetation was induced in 24 New Zealand White rabbits according to the classical model of Durack and Beeson and currently developed in our group. In brief, a polyethylene catheter (PE50, Clay Adams) was connected to a manometer and inserted into the left ventricle through the right carotid artery, under ketamine-xylazine anesthesia. The ventricular location of the catheter was checked by angioscopy. The catheter remained indwelling throughout the experiment. Because right-sided catheterization was not possible, bacterial inoculations were performed 5 days after catheterization. Right-sided thrombotic vegetations were induced in 13 rabbits by right heart catheterization in the rabbit. Right-sided thrombotic vegetations were induced in 7 rats with the use of a Silastic catheter. Bacterial inoculation was performed in 7 rats via the left jugular vein under halothane anesthesia. Right-sided thrombotic vegetations were similar in 7 rats with the use of a Silastic catheter. All of the rats were inoculated with S aureus.

### Microbial Strains

We used 5×10^7 colony-forming units of E faecalis JH2-2 and 2.5×10^6 colony-forming units of S aureus HM 1054 as inocula in 1 mL 0.9% saline. Rabbits were inoculated with 1 mL and rats with 0.5 mL of the suspension. Mean bacterial counts in vegetations were close to 8 log colony-forming units per gram whatever the animal species and the bacterial strain used.

### Scintigraphy

#### 99mTc-ANX Labeling Procedure

Sodium pertechnetate (99mTcO₄⁻, 1480 MBq, freshly eluted) and 50 μg stannous tricine buffer (pH 6) were added to a vial containing 0.275 mg recombinant human Hynic-Annexin V (National Cancer Institute BRB Preclinical Repository, Frederick, Md) and left to incubate for 15 minutes at room temperature. The quality control was performed with instant thin-layer chromatography developed in acid citrate dextrose buffer. The radiochemical purity was always superior to 88%.

#### 99mTc-ANX Scintigraphy

All acquisitions were performed with the use of a dedicated small-animal γMAGER-S system (Biospace Mesures, France)
equipped with parallel low-energy, high-resolution collimators, 256×256 matrix, and 15% energy window centered on 140 kEV.

In vivo scintigraphy was performed under intravenous (rabbits) or intraperitoneal (rats) pentobarbital anesthesia (40 mg/kg body wt; Ceva Santé Animale, France). Thirteen minutes after intravenous injection of 74 MBq/kg 99mTc-ANX, a 30-minute planar acquisition centered on the head was performed, followed by a 60-minute planar acquisition centered on the chest (ventral position) in rabbits. In rats, a 30-minute planar acquisition centered on the chest was followed by dual-head tomographic scintigraphy for 30 minutes. Additionally, a helicoidal computed tomography scan (μCT, Biospace Mesures, France) was performed simultaneously to scintigraphy in rats with right-sided endocarditis for image coregistration. All animals were euthanized after in vivo scintigraphic recording. The heart, lungs, and brain were rapidly excised, washed briefly, and placed under the gamma camera for ex vivo acquisition.

Data Analysis
Scintigrams were assessed visually to determine the presence of a focal 99mTc-ANX uptake in the heart, lungs, and brain. For in vivo quantification, the ratio between the activity (mean counts per second per square millimeter) of the vegetation and the underlying background activity (ratio of vegetation to background) was calculated. For background activity, the region of interest was drawn immediately underneath the vegetation activity to take into account both lung and spine uptakes. For ex vivo quantification, the ratio between the activity of the vegetation and the activity of a region of interest drawn on normal myocardium remote from the vegetation (ratio of vegetation to remote myocardium) was used.

Quantitative Autoradiography
The heart, lungs, and brain were dissected carefully, and the endocardial vegetations and valvular, vascular, and myocardial surrounding tissues were removed, frozen, and cut into transverse sections of 20-μm thickness, which were exposed in a digital radioimager (Instant Imager, Packard, Meriden) for 20 hours. The activity normalized to the region of interest area (mean counts per minute per square millimeter corrected for background activity) was determined on autoradiograms of sections of vegetations, emboli, and myocardium remote from vegetations. Quantification was performed by calculating the ratio between the activity of the vegetation and the activity of a region of interest drawn on normal myocardium remote from the vegetation (ratio of vegetation to remote myocardium). Quantification was not performed in sham-operated animals because it would have been hampered by a partial volume effect due to the thickness of the valve. According to calibration studies previously reported, with activity standards of tissue-equivalent homogenates, 50 counts per minute per square millimeter of 99mTc-ANX approximated 210 kBq/mg in autoradiography.

Histology
Some representative samples of left- and right-sided endocarditis vegetations in rabbits and rats, including aortic tissue, aortic valves, superior vena cava, right atria, tricuspid and pulmonary valves, and left and right ventricles, were fixed in paraformaldehyde for 24 hours, embedded in paraffin for morphological analysis, or frozen in OCT for cryostat sectioning and immunohistochemistry. Five-micrometer-thick serial sections were routinely stained with Masson’s trichrome to visualize erythrocytes and fibrin, with hematoxylin-eosin for cells and nuclei, with Alican blue coupled with nuclear red to reveal areas of mucoid accumulation and their relation to cell nuclei, with orcein for elastin, and with Sirius red for collagen.

To localize dying cells, the terminal transferase-mediated dUTP nick end-labeling (TUNEL) method was used on fixed sections according to the manufacturer’s instructions (Roche). A positive control with DNase I (Qiogene) treatment (3 U/mL) and a negative control omitting the use of terminal transferase were performed simultaneously. Cell nuclei were shown by counterstaining with 100 ng/mL 4',6'-diamidino-2-phenylindole hydrochloride (DAPI). Biotinylated annexin V (Beckman Coulter), which binds phosphatidylserine, was used to probe for in situ anionic phospholipids with subsequent detection by avidin/biotin complexes conjugated with horseradish peroxidase (HRP) and revealed by the 3,3'-diaminobenzidine (DAB) reaction. Goat anti-mouse P-selectin antibody (SC 6943, Santa Cruz Biotechnology, Santa Cruz, Calif) (1/50) was used to localize activated platelets on cryostat sections and revealed by an anti-goat antibody conjugated with HRP, followed by the DAB reaction.

Cryostat sections were used for autoradiography and then stained by Masson’s trichrome, and the full sections were reconstituted under the microscope with the use of Cartograph software (Microvision, France). Superposition of both images at the same scale was

Figure 2. Morphology of aortic valve endocarditis in rabbit. A, Macroscopic view of the vegetation attached to the valve (arrow 1) is shown. The catheter path along the aortic wall was also the site of thrombus formation (arrow 2). The coronary artery orifice was not obliterated (arrow 3). Microscopic view of a rabbit aortic valve (Masson’s trichrome, magnification ×4) is also shown: a normal valve (B); after aseptic catheterization, showing the presence of a small thrombus on the valve (arrows) (C); and septic vegetation (arrow) attached to the valve (arrowhead) (D).
then processed (fusion images) to localize $^{99m}$Tc-ANX uptake (autoradiography) on histological sections.

**Statistical Analysis**

Continuous variables are expressed as mean±SEM. Unless otherwise specified, pooled data obtained in rabbits and rats were compared by a 2-factor ANOVA (infected/noninfected and rabbits/rats). The activity ratios in infected, noninfected, and sham-operated rabbits were compared with an ANOVA followed by the Scheffé post hoc test. Linear regression and Pearson correlation coefficients were performed, separately for each species, to relate $^{99m}$Tc-ANX vegetation-to-background activity ratios obtained by in vivo and ex vivo scintigraphy and autoradiography.

In the paragraph entitled “Impact of Bacteria Strain,” because of the low number of observations, the $^{99m}$Tc-ANX uptake between *S. aureus* and *E. faecalis* groups was compared by use of the Mann-Whitney *U* test, and the incidence of pulmonary emboli between *S. aureus*, *E. faecalis*, and noninfected groups was compared by use of the Kruskal-Wallis test. The level of significance was set at $P<0.05$.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

**Results**

All the animals in which the catheter was correctly positioned within the ventricles developed aseptic or septic vegetations. Macroscopic examination at time of euthanasia showed that left-sided vegetations were localized mainly on the aortic valves but could also be observed on the aortic wall along the catheter path (Figure 2A) and in the ventricle, in close contact with the catheter tip.

**$^{99m}$Tc-ANX Functional Imaging**

**Left-Sided Endocarditis**

Left heart catheterization gave similar results in rabbits and rats. Whereas sham-operated animals presented a homogeneous...
background activity in heart and lung area, catheterized animals without bacterial inoculation presented a focal $^{99m}$Tc-ANX uptake in the aortic valve area. Bacteria-inoculated animals presented a more intense uptake in the aortic valve area (Figure 3), and frequent accessory foci localized in the ascending aorta and in the left ventricle in contact with the catheter tip. Quantification of in vivo $^{99m}$Tc-ANX uptake on planar scintigraphy showed a higher ratio of vegetation to background activity in bacteria-inoculated animals than in those without bacteria inoculation (Figure 4A) and higher ratios in rats compared with rabbits in both infected and noninfected animals (rats infected, 2.2±0.1; rabbits infected, 1.6±0.07; rats noninfected, 1.6±0.1; rabbits noninfected, 1.2±0.05; ANOVA $P<0.0001$ for both infection and species factors). The uptake ratio in sham-operated rabbits was close to 1 (1.0±0.02), significantly lower than in noninfected and infected rabbits (ANOVA $P=0.002$ and $P=0.0001$, respectively).

Similar results were obtained by ex vivo imaging of explanted hearts, and both were correlated ($R^2=0.8$, $P<0.0001$ in rabbits and $R^2=0.6$, $P=0.05$ in rats), indicating a low impact of background activity. On autoradiography, the ratio of vegetation to remote myocardium was higher in infected animals than in noninfected animals (Figure 4B), but, conversely to in vivo imaging, the ratio was independent of the animal species ($P=0.9$ for the species factor). Of note, there was no threshold value allowing differentiation between these 2 groups. Quantification of $^{99m}$Tc-ANX uptake by the vegetation in vivo and quantification by autoradiography were also correlated (Figure 4C). No focal uptake was detected in the brain either by in vivo or ex vivo scintigraphy or with autoradiography (acquisitions performed only in rabbits; data not shown).

**Impact of Bacteria Strain**

The impact of the bacteria strain on $^{99m}$Tc-ANX uptake has been assessed in vivo in the rabbit model. There was no significant difference in $^{99m}$Tc-ANX uptake between $S$ aureus and $E$ faecalis whether the vegetation was left-sided (in vivo vegetation/background ratio, 1.5±0.1 versus 1.5±0.2, respectively; $P=1$) or right-sided (in vivo vegetation/background ratio, 1.7±0.5 versus 1.6±0.4, respectively; $P=0.8$). However, lesions (vegetations and emboli) showed a more rapid onset with $S$ aureus.

The main difference lied in the incidence of pulmonary emboli in right-sided endocarditis, ranging from 11 to 17 (median, 12) in rabbits infected with $S$ aureus versus 1 to 4 (median, 1) in those infected with $E$ faecalis and 0 to 1 (median, 1) in those not infected ($P=0.007$ for $S$ aureus group versus 2 other groups).

**Microscopic Morphology of Endocardial Thrombotic Vegetations**

Microscopically, sham-operated rabbits presented a normal aspect of the aortic valve (Figure 2B). Chronic catheterization without microbial inoculation caused the development of small thrombi on the aortic valve (Figure 2C). In contrast, septic vegetations were florid thrombi (Figure 2D) proliferating on the valves and along the catheter path.

Histologically, vegetations had the usual aspect of a platelet-fibrin–rich clot (Figure 7), including a network of fibrin (Mallory staining) and the accumulation of red blood cells and leukocytes. Similar lesions were observed in the aorta, vena cava, and ventricle. Annexin V immunostaining predominated in the luminal pole of the vegetations (Figure 7E). Platelet-dependent P-selectin expression was also limited to the luminal pole of the vegetations, at the interface with circulating blood (Figure 7F). The histological aspect was similar in both species and in left- and right-sided vegetations.

The most interesting and novel histological aspect was observed with Alcian blue staining. Alcian blue staining revealed the presence of large positive areas of mucoid substance in the valvular, aortic, and myocardial tissue in close contact with the vegetations (Figure 8A and 8B). These mucoid areas were
devoid of cell components, as demonstrated by nuclear red counterstaining. These areas were the site in which TUNEL positivity was localized (Figure 8C), suggesting that mesenchymatous cell disappearance was topographically linked to vegetation activities. In contrast, at this stage, the extracellular matrix was still conserved, suggesting that cell disappearance preceded matrix degradation in these experimental models of endocarditis. The histological aspect is similar in right-sided endocarditis and in both rabbits and rats. Pulmonary emboli were characterized by the presence of thrombi obliterating small arterioles, showing great similarities with endocarditic vegetations.

**99mTc-ANX Uptake and Vegetation/Host Tissues Interface**

Compared analysis of autoradiographies and corresponding histological sections of cardiac tissues showed that **99mTc-ANX uptake was largely predominant on the vegetation and, more precisely, at its luminal pole in contact with the blood pool (Figures 3C and 6E).** A similar pattern was observed in ventricular wall lesions, but to a lesser extent because of the smaller vegetation size, and in phlebothrombosis associated with the Silastic catheter path in the vena cava (Figure 6F). The underlying tissue also exhibited visible **99mTc-ANX uptake,** although much fainter than that of the vegetations themselves, corresponding to mucoid areas detected by Alcian blue staining and apoptotic cells shown by TUNEL.

Compared analysis of autoradiography and corresponding histological sections of lung tissues showed that **99mTc-ANX uptake matched with embolized thrombi in pulmonary arterioles (Figure 5).**

**Figure 6.** Aortic valve endocarditis (*Enterococcus*) in rat: In vivo scintigraphy shows a high focal uptake of **99mTc-ANX** in the aortic valve area (arrow) observed on both planar (A) and tomographic (B) acquisitions (L indicates liver uptake of the tracer). Tricuspid valve endocarditis (*Staphylococcus*) in rat: In vivo scintigraphy shows uptake foci in the tricuspid (solid arrow) and pulmonary (dotted arrow) valve area seen on both planar (C) and tomographic (D) (coregistered with computed tomography) acquisitions. Also shown are histology, autoradiography, and the fusion image (autoradiography appears in yellow) of the vegetation (solid arrows) attached to the tricuspid valve (dotted arrow) (E) and the superior vena cava phlebothrombosis indicating the localization of **99mTc-ANX** activity on the thrombus (solid arrows) sheathing the catheter pathway (dotted arrow) (F).

**Figure 7.** Histological aspect of aortic valve vegetations in rabbits (Masson’s trichrome, magnification ×10) (A); an aortic valve vegetation in the rat (hematoxylin-eosin, magnification ×10) (B); a vegetation along the aortic wall in rabbit (Mallory staining of fibrin, magnification ×10) (C); disappearance of smooth muscle cells and mucoid degeneration in the aortic wall underlying the vegetation (Alcian blue, magnification ×20) (D); annexin V (peroxidase/DAB) staining on the luminal pole of the vegetation (magnification ×100) (E); and P-selectin (peroxidase/DAB) staining on the luminal pole of the vegetation (magnification ×100) (F).
discussion

As proposed by some authors and shown in the present study, nonbacterial and bacterial thrombotic endocarditis share common pathological consequences, mainly linked to platelet procoagulant and proteolytic activities conveyed by thrombus formation and degradation, which are greatly amplified by biological activities conveyed by the bacteria themselves. Therefore, whatever their predominant source, platelet proaggregant and procoagulant activities participate in the development of vegetations, fibrinolytic activities contribute to sepsis diffusion and embolic potential, and numerous proteases are involved in tissue destruction in relation to their ability to damage extracellular matrix and to induce cell death.

Bacteria–platelet interactions play a critical role in the pathogenesis of infective endocarditis. Direct or indirect binding of bacteria to platelets can provoke their activation and aggregation, resulting in fibrin formation. During activation, aggregation, and disintegration, platelets expose negatively charged phosphatidylserines, which are necessary for the assembly of tenase and prothrombinase complexes and thrombin generation, at the interface with circulating hemostatic blood components. Therefore, platelet-exposed phosphatidylserine is the biological link between platelet activation and fibrin clot formation. Annexin V specifically binds with nanomolar affinity to phosphatidylserines. Thus, radiolabeled annexin V has been used previously for in vivo scintigraphy of apoptotic cells in both animals and humans and acute platelet-rich or chronically renewed mural thrombi, maintaining a permanent interface with the circulating blood. Therefore, exposed phosphatidylserines can be targeted by $^{99m}\text{Tc}-\text{ANX}$ to obtain functional imaging of platelet-fibrin–rich vegetations in endocarditis. Our results provide evidence of this potential, showing that in 2 species (rabbit and rat), with the use of strains from 2 different bacterial species ($\text{Enterococcus}$ and $\text{Staphylococcus}$), $^{99m}\text{Tc}-\text{ANX}$ binds to and allows in vivo detection of right- and left-sided thrombotic vegetations in experimental endocarditis. Superposition of autoradiography and corresponding histological section showed that, as in the aseptic mural thrombus in abdominal aortic aneurysms, annexin V predominantly binds to the most luminal layer of the vegetation, corresponding to the dynamic biologically active interface between the circulating blood components and the vegetation. Because the Silastic catheter used for right-sided endocarditis is silicone rubber, as human pacemaker leads, imaging of phlebothrombosis associated with the venous catheter path is also of interest in detecting the extent of the septic-thrombotic process.

Enhancement of the proaggregant and procoagulant potential of the vegetation by bacterial colonization had been suggested in our study by the significantly higher uptake of $^{99m}\text{Tc}-\text{ANX}$ by septic vegetations compared with aseptic ones (nonbacterial thrombotic endocarditis). A procoagulant state has been associated with an increased risk of embolism in infective endocarditis.

Detection of Embolization

$^{99m}\text{Tc}-\text{ANX}$ permitted visualization not only of the endocardial vegetations but also of peripheral emboli, which are an important prognostic determinant in endocarditis. This ability was demonstrated by the presence of focal $^{99m}\text{Tc}-\text{ANX}$ uptake in lungs associated with right-sided endocarditis. These signals were obtained with $\text{Staphylococcus}$ as well as with $\text{Enterococcus}$, providing evidence of the common pathway of platelet phosphatidylserine exposition in vegetation-induced peripheral emboli. However, the incidence of emboli was much greater when the infective strain was $\text{S. aureus}$, which is known to interact with the plasmaminergic system of the host.

Nevertheless, we did not succeed in detecting emboli in experimental left-sided endocarditis. This could be explained by the short survival rate of staphylococcal-induced endocarditis compared with right-sided endocarditis.

Underlying Cardiac Tissue

Finally, in addition to that detected at the luminal interface of the vegetation, limited but significant uptake of $^{99m}\text{Tc}-\text{ANX}$ could
also be observed in tissue areas underlying the vegetations (Figure 6E). These sites of uptake always corresponded to areas of predominant apoptosis as evidenced for the first time by TUNEL and Alcian blue staining, showing areas of myocyte disappearance and replacement by mucoid degeneration. Such basophilic areas, corresponding to the accumulation of modified glycosaminoglycans, are usually described in other noninfective vascular pathologies such as aneurysms and dissections of the ascending aorta and also in noninflammatory tendinopathy, including that of the mitral chordae. In these cases, mucoid degeneration appears to be linked to cell disappearance and matrix degradation. In the present study, we constantly observed such a phenomenon of cell disappearance and mucoid retention in the tissues immediately underlying the vegetations. In contrast, probably because of the short delay of observation (3 to 5 days), we did not observe gross evidence of matrix degradation in these areas, suggesting that cell disappearance and glycosaminoglycan retention preceded matrix degradation. In endocarditis, this observation could be linked to the ability of proteases released by the septic vegetation to induce detachment and death of myocytes. Streptococcal and staphylococcal strains convey plasminogen activators (streptokinase and staphylokinase), leading to the generation of plasmin, which in turn may activate host proteases. In addition, the bacteria themselves may secrete cell toxin and various proteases, such as metalloproteinases, serine, and cysteine proteases, which are involved in thrombogenesis and fibrinolysis. This proteolytic activity in the vicinity of the vegetation has 2 consequences: one is to promote lysis of the vegetation itself and dissemination of septic emboli, and another is to promote degradation of the extracellular matrix and invasion of the underlying tissue, leading to valvular damage and/or abscess formation.

In conclusion, our present study demonstrated that 99mTc-ANX can be used for detecting thrombotic vegetations in experimental endocarditis, an observation that was recently confirmed in a case report in humans. Moreover, 99mTc-ANX scintigraphy also could help to localize peripheral emboli and thus could probably be used to evaluate therapeutic efficacy. 99mTc-ANX uptake results from 2 different mechanisms depending on the vegetation/host tissue interface: On the luminal pole of the vegetation, it reflects phosphatidylerine exposition mainly by activated platelets, whereas on the abluminal pole, it reflects myocyte death by apoptosis. The former mechanism is very intense and permits noninvasive imaging of endocardial thrombotic vegetations. The latter is only faint, corresponding to areas of mucoid degeneration, providing evidence of the initial tissue injury induced by proteases released by the vegetation.

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Disclosures
None.

References

**CLINICAL PERSPECTIVE**

The mural thrombus, whatever its location (vascular, intracardiac, or valvular), exhibits biological activity (proaggregant and fibrinolytic) because of the maintenance of a dynamic interface with the circulating blood. Experimental thrombotic endocarditis is a model of mural thrombus, in which biological activity is enhanced by bacterial colonization. Binding of bacteria to platelets can induce their activation and aggregation, resulting in phosphatidylserine exposure. Annexin V specifically binds with nanomolar affinity to phosphatidylserines. This preclinical study shows the ability of radiolabeled annexin V to provide in vivo functional imaging of platelet activation in experimental endocarditis. Annexin V uptake was predominant in the luminal layer of the vegetation, at the interface with circulating blood, and its uptake was enhanced by bacterial colonization of the vegetation. As suggested by previous preclinical studies of atherothrombosis or abdominal aortic aneurysm models, radiolabeled annexin V is able to provide insight into platelet activation in various degenerative cardiovascular diseases. Additionally, radiolabeled annexin V allowed the detection of pulmonary emboli in right-sided endocarditis. The embolic potential of vegetations is related to their fibrinolytic activity and is a prognostic determinant in human endocarditis. Indeed, the incidence of emboli was high when the infective pathogen was *Staphylococcus aureus*, a bacterium known to interact with the plasminergic system of the host. In this regard, tracers targeting fibrinolytic activity would be of interest. Finally, the detection of phlebothrombosis associated with silicone rubber catheters, close to human pacemaker leads, suggests a potential role of radiolabeled annexin V in the diagnosis of implantable cardiac device infection.
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