The particle gel immunoassay as a rapid test to rule out heparin-induced thrombocytopenia?

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Heparin-induced thrombocytopenia (HIT) is a prothrombotic condition characterized by a platelet decrease of 50% or thrombosis with a temporal relationship of 1 to 2 weeks after the initiation of heparin. These surrogate markers are useful for the clinical assessment¹ but are hardly applicable in multimorbid patients with clinical conditions that mimic HIT. Platelet activation assays (heparin-induced platelet activation [HIPA] and serotonin release assay) and platelet factor 4 (PF4)/polyanion enzyme-linked immunosorbent assay (ELISA) confirm HIT.² HIPA and serotonin release assay are highly specific, but they are laborious and require selected donor platelets and extended experience. High titer immunoglobulin (Ig)G antibodies correlate with clinical HIT,³ but ELISA is also time-consuming. The alternative heparin/PF4-antigen particle gel immunoassay (PaGIA) is easy, provides results within 1 hour, and detects mainly IgG but also IgA/M antibodies. We evaluated the specificity and sensitivity of PaGIA in relation to HIPA and ELISA in 285 patients with an undetermined likelihood for HIT with the intention to validate it as a rapid assay to exclude HIT.

PATIENTS AND METHODS

Samples from 285 consecutive patients (median 71 years, range 1–97 years, 45% cardiovascular surgery; female/male 56/73, and 55% medical, female/male 78/78) and 89 healthy controls (median 42 years, range 26–64 years; female/male 49/40) were tested prospectively. The PaGIA (ID-PaGIA H/PF4, DiaMed, Cressier s/Morat, Switzerland) was performed as described⁴ and evaluated by 2 independent technicians. Briefly, heparin/PF4-coated red particles become crosslinked in the presence of heparin antibodies and remain on top of a gel chamber after centrifugation. In the absence of antibodies, all particles sediment to the bottom of the gel chamber. A combined anti-human IgG/A/M and anti-human IgG only conjugate were used for the PF4-ELISA (PF4-ENHANCED; GTI, Waukesha, Wis) following the manufacturer's instructions. HIPA was performed as published.⁵ Analyses include chi-square, Mann-Whitney, receiver operating characteristic (ROC) curves (HIPA vs ELISA to deter-

mine their cutoff values), Bayes' theorem, and logistic regression. Data are presented as median and range.

RESULTS

HIPA was positive in 12% of patients. On the basis of ROC curves (ELISA vs HIPA), optical density (OD) cutoff values for the IgG/A/M-ELISA and IgG-ELISA were 0.761 and 0.564, respectively. In controls, the OD value of the IgG-ELISA was 0.066 (0.028–0.500) and lower than from patients' samples, which were negative by PaGIA and IgG/A/M-ELISA ($n = 158$; 0.074; 0.009–0.339; $P = .017$). Of note, 2 male controls who had never received heparin had IgG-ELISA OD values of 0.425 and 0.500. The latter serum was also positive by PaGIA, but both were HIPA negative. The OD values of the other 87 controls were less than 0.180.

PaGIA was positive in 70 patients (25%), and the relation to HIPA and ELISAs is shown in Table 1. In both patient groups, both ELISA ODs were higher if PaGIA and IgG/A/M-ELISA were positive compared with negative PaGIA but positive IgG/A/M-ELISA ($P < .001$). The frequency of PaGIA-positive samples was similar among surgical and medical patients in all OD ranges (Figure 1). Three PaGIA-negative samples were HIPA positive, and one was IgG-ELISA positive.

On the basis of ROC curves (ELISA vs HIPA), IgG/A/M-ELISA (OD cutoff 0.761) and IgG-ELISA (OD cutoff 0.564) had sensitivities of 81% and 86%, and specificities of 75% and 81%, respectively (positive and negative predictive values were 31% and 97% and 39% and 98%, respectively, for IgG/A/M-ELISA and IgG-ELISA). Specificity, sensitivity, positive predictive value, and negative predictive value of PaGIA based on HIPA and ELISA are shown in Table 2. Logistic regression analysis showed that PaGIA, IgG/A/M-ELISA, and IgG-ELISA were significant predictors for the results of HIPA ($P < .001$) and that the IgG-ELISA had the highest explained variance (41.2%). There were no differences between male and female patients concerning specificity and sensitivity of any test.

DISCUSSION

The high mortality associated with HIT led to an increasing demand for its laboratory exclusion in patients with various clinical conditions that mimic HIT, particularly because multimorbid patients are more likely to form PF4 antibodies.² The clinical score is highly reliable to exclude

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TABLE 1. Optical density values of the immunoglobulin-G/A/M enzyme-linked immunosorbent assay and immunoglobulin-G enzyme-linked immunosorbent assay in surgical and medical patients in relation to the results of the particle gel immunoassay

	PaGIA	IgG/A/M ELISA	OD IgG/A/M ELISA	OD IgG ELISA	
			Median (range)	Median (range)	
Surgical patients n = 129	Positive 28	Positive 28	1.813 (0.406–3.000)	1.007 (0.112–3.000)	] $P < .001$
		Negative 0	—	—	
	Negative 101	Positive 15	0.481 (0.407–1.604)	0.270 (0.046–1.481)	
		Negative 86	0.154 (0.060–0.395)	0.068 (0.021–0.339)	
Medical patients n = 156	Positive 42	Positive 36	1.806 (0.456–3.000)	1.480 (0.061–3.000)	] $P < .001$
		Negative 6	0.157 (0.114–0.339)	0.117 (0.044–0.233)	
	Negative 114	Positive 42	0.520 (0.402–1.736)	0.242 (0.048–2.028)	
		Negative 72	0.181 (0.050–0.397)	0.076 (0.020–0.313)	

PaGIA, Particle gel immunoassay; Ig, immunoglobulin; ELISA, enzyme-linked immunosorbent assay; OD, optical density.

FIGURE 1. Distribution of surgical and medical patients with positive and negative PaGIA results in 3 OD intervals of the IgG-ELISA. The frequency of PaGIA-positive and negative samples was similar among surgical and medical patients in all OD ranges (<0.564, $P = .1$; 0.564–1.000, $P = .3$; >1.000, $P = 1.0$). PaGIA, Particle gel immunoassay.**TABLE 2.** Relation of the particle gel immunoassay to heparin-induced platelet activation test and to immunoglobulin-G/A/M and immunoglobulin-G enzyme-linked immunosorbent assay

	PaGIA vs HIPA		PaGIA vs IgG/A/M-ELISA		PaGIA vs IgG-ELISA	
	Surgical	Medical	Surgical	Medical	Surgical	Medical
Sensitivity %	71	79	92	73	86	73
Specificity %	68	75	94	89	92	85
PPV %	21	32	79	69	67	57
NPV %	95	96	98	90	97	92

PaGIA, Particle gel immunoassay; HIPA, heparin-induced platelet activation test; Ig, immunoglobulin; ELISA, enzyme-linked immunosorbent assay; PPV, positive predictive value; NPV, negative predictive value.

HIT when clinical features are unambiguous, but a rapid assay is desirable for patients with undeterminable probability for HIT. Two commercialized assays provide results within 1 hour: the PaGIA and PIFA (Akers Biosciences, Thorofare, NJ). Reports to the Platelet Immunology Scientific Subcommittee (Geneva 2007) suggest a low specificity for the PIFA.

According to our results, the PaGIA can serve as a rapid test to exclude HIT because a negative PaGIA is rarely associated with high titer IgG PF4 antibodies or a positive HIPA.

Notably, PaGIA is negative in rare non-PF4 antibody-mediated HIT.⁵ Alternative anticoagulation should be considered until results from functional testing are available if the PaGIA is positive and in patients with a high probability for HIT and a negative PaGIA.

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A potential of autologous pericardium for a sustained-release carrier of vancomycin: A pilot study in vitro

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Autologous pericardium has been widely used to repair destructed annuli in infective endocarditis complicated by annular abscess.^{1,2} In the present study we investigated the in vitro property of autologous pericardium for a sustained-release carrier of vancomycin.

CLINICAL SUMMARY

Between January and May 2002, autologous pericardium with pericardial fat was harvested from the patients (n = 6) who underwent cardiac operations in our institute. After harvesting, the pericardium was cut into 7 pieces (1 cm × 1 cm each). Then 0.2 mL of vancomycin solution (15 mg/mL) was dropped onto each pericardium and incubated for 1 hour at room temperature so that the vancomycin solution was completely absorbed into the pericardium. The 7 pericardial patches with vancomycin were soaked in 7 test tubes containing 5 mL of saline, respectively. The test tubes were placed in the shaker and kept at 37°C. At 6 hours, 12 hours, and 1, 3, 5, 7, and 10 days after the incubation, the pericardium and the saline samples were collected and frozen from 1 of the 7 test tubes, and the saline in the residual test tubes were replaced with 5 mL of fresh saline (ie, the pericardia were left uncollected), respectively. We replaced the saline in the residual test tubes at each time point to maintain the

diffusion gradient between the pericardium and the saline. To prevent the degradation of the samples, we froze them until the concentrations of vancomycin were measured. The vancomycin concentrations of the pericardium and the saline samples were measured as previously described.^{3,4} We obtained written informed consent from each patient after a full explanation of this study. The protocol of this study complied with the principles set forth in the Helsinki Declaration.

All values are expressed as means ± standard deviations. Figure 1 shows that the percentage reaming of vancomycin in the pericardium at each time point was 67.8% ± 17.8%, 53.8% ± 12.3%, 37.8% ± 9.8%, 25.1% ± 10.1%, 12.6% ± 3.3%, 7.1% ± 3.1%, and 4.3% ± 1.7% for 6 hours, 12 hours, and 1, 3, 5, 7, and 10 days after the incubation, respectively. Figure 2 shows that the concentrations of vancomycin in the saline samples were 789 ± 143 µg/mL, 376 ± 56 µg/mL, 144 ± 23 µg/mL, 56 ± 14 µg/mL, 38 ± 5.2 µg/mL, 27 ± 3.8 µg/mL, and 15 ± 2.7 µg/mL for 6 hours, 12 hours, and 1, 3, 5, 7, and 10 days after the incubation, which were all greater than the minimum inhibitory concentration of vancomycin (2.0 µg/mL) against methicillin-resistant *Staphylococcus aureus* (MRSA). These results indicate that the pericardium can slowly release vancomycin and maintain the minimum inhibitory concentration of MRSA around the pericardium for more than 10 days.

DISCUSSION

In the present study we found that the autologous pericardium with fat might have a potential for a sustained-release carrier of vancomycin. Although this is an in vitro study and the mechanism of the sustained release was unclear, the property might help prevent prosthetic valve endocarditis by MRSA after reconstruction of the infected annulus.

Antibiotics are usually administrated systematically to prevent all forms of infection; however, this might be

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