PRELIMINARY NOTE

Methods of Measuring Splenic Blood Flow and Platelet Transit Time with In-111-Labeled Platelets

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Following bolus intravenous injection of In-111-labeled autologous platelets in humans, splenic blood flow (SBF) was measured by compartmental analysis of the equilibration of platelets between blood and the intrasplenic platelet pool. The result correlated closely ($r = 0.94$, $p < 0.001$) with SBF measured from analysis of the first-pass time-activity curve recorded over the spleen by external detection. The intrasplenic platelet transit time (t_p), measured concurrently with SBF from compartmental analysis, correlated closely ($r = 0.78$, $p < 0.001$) with t_p measured from deconvolution analysis. These correlations appear to support the use of compartmental analysis of the equilibration of radiolabeled platelets between blood and the intrasplenic platelet pool as a valid technique for the simultaneous measurement of SBF and t_p. Both of these parameters have potential clinical usefulness for the understanding of intrasplenic platelet kinetics in a variety of disorders.

J Nucl Med 25: 86–90, 1984

We recently described a noninvasive technique for measuring splenic blood flow (SBF) using autologous platelets labeled with indium-111 (*1*,*2*). The technique, based on the kinetics of intrasplenic platelet pooling (*3*,*4*) utilizes two parameters: (a) the rate constant of equilibration of radiolabeled platelets between the circulating blood and intrasplenic platelet pool following peripheral bolus injection; and (b) the partition fraction of radiolabeled platelets between these two compartments at equilibrium. In addition to SBF, obtained in units of percent total extrasplenic blood volume (TBV) per min, the technique also yields mean intrasplenic platelet transit time (t_p).

In addition to the compartmental analysis described above, other analytical techniques for the calculation of SBF and t_p after the injection of In-111-labeled platelets are possible. The aims of this paper are to compare SBF and t_p, as calculated from compartmental analysis, with SBF and t_p based on alternative analytical approaches. Since the latter—analysis of the first-pass splenic time-activity curve for SBF and deconvolution analysis (*5*) for t_p—are based on different assumptions, such a comparison offers validation of compartmental analysis as a method of measuring SBF and t_p.

Received Feb. 25, 1983; revision accepted Aug. 9, 1983.

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METHODS

Patients. The subjects were referred to the department of radiology for investigations based on radiolabeled platelets. Their clinical conditions varied considerably. Patients with overt cardiac disease were excluded from this study because in most of them it was difficult to identify clearly an early plateau on the first-pass time-activity curve (see below). Patients with very short platelet life spans (<1 day) were also excluded, since it could not be assumed that platelet destruction in the spleen was negligible over the time course of In-111-platelet equilibration (see below).

Platelet labeling. Platelets were harvested from 20–50 ml whole blood obtained by venepuncture using a 19-guage butterfly. The larger volume was taken from patients with thrombocytopenia. In two severely thrombocytopenic patients (platelet counts of less than $20{,}000/\mu l$; $20 \times 10^9/l$), ABO and rhesus-matched donor platelets were used. The platelets were labeled with In-111 acetylacetonate as previously described (*6*,*7*), with

efficiency 50–90%. The injected dose was 100–200 μCi in a volume of less than 5 ml of plasma. In some subjects platelet survival was monitored by taking periodic blood samples following reinjection of In-111 platelets. Cell-bound activity was measured in these samples by subtraction of plasma activity.

Dynamic imaging. With the subject lying supine, the radiolabeled platelets were rapidly injected into an antecubital vein. A gamma camera, on line to a computer, was positioned posteriorly over the chest and upper abdomen and dynamic imaging performed. Before injection, a frame time of 1 min for 2 min was used for background acquisition; then of 5 sec for 90 sec, and then of 1 min for 30 min. Blood samples of 5 ml were taken before platelet injection from the arm opposite the injection site, and at 3 and 30 min after injection.

DATA ANALYSIS

Splenic blood flow (SBF). *Compartmental analysis.* SBF/TBV was calculated as described previously (*1*,*2*). Briefly, the rate constant (K_s) of equilibration of In-111 platelets between the splenic platelet pool and circulating blood was calculated by a computerized maximum-likelihood estimate from the uptake curve recorded over the spleen from 0 to 30 min.
Then

$$K_s = K_1 + K_2 \text{ (see Appendix)}, \quad (1)$$

where K_1 is the rate constant of input into the spleen (i.e. SBF/TBV) and K_2 the output rate constant (i.e. the reciprocal of t_p).
At equilibrium,

$$\frac{K_1}{K_2} = \frac{Q_s}{Q_b} \text{ (see Appendix)}, \quad (2)$$

TABLE 1. VALUES OF SBF (MEAN ± s.e.m.) CALCULATED BY COMPARTMENTAL ANALYSIS IN SUBJECTS WITH AND WITHOUT SPLENOMEGALY*

Diagnois (No. of subjects)	Spleen size	
	Normal	Enlarged
Normal (3)	3.3, 3.3, 4.8	—
Rheumatoid arthritis (13)	4.5 (± 0.8)	12.3 (± 2.3)
Systemic lupus erythematosus (2)	8.0, 10.5	—
Other immune complex diseases (6)	7.0, 8.2	16.9 (± 2.9)
Scleroderma (1)	4.9	—
Polymyositis (1)	4.4	—
Portal hypertension (1)	—	11.5
Primary polycythemia (1)	4.3	—
Coronary atherosclerosis (3)	4.2, 5.3, 5.3	—

* Units: percent TBV per min.

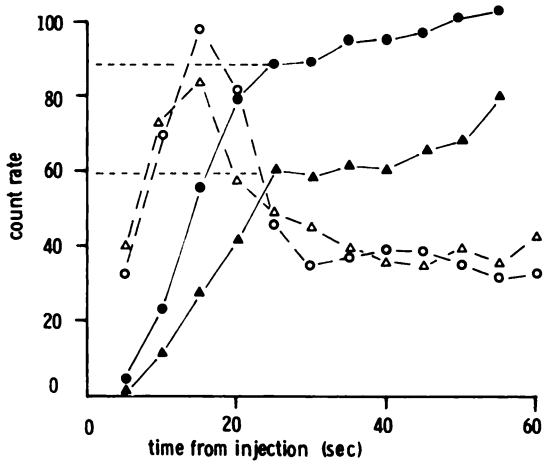

FIG. 1. Time-activity curves recorded over spleen (closed symbols) and cardiac blood pool (open symbols) following bolus intravenous injection of In-111-labeled platelets. Count rate in arbitrary units. Triangles and circles correspond to different subjects. Horizontal dashed lines correspond to first-pass splenic plateau (see text).

where Q_s and Q_b are, respectively, the quantities of In-111 platelets in the spleen and circulating blood at equilibrium. Q_s/Q_b was calculated from the time-activity curve recorded over the cardiac blood pool, making the assumption that In-111 platelets were distributed only between circulating blood and spleen, as in a closed two-compartmental, well-mixed system. There was some liver uptake (Q_h) which accounted for about 12% of the dose (*8*) but it was completed before 6 min, and therefore corrected for by an extrapolation procedure as previously described (*1*,*2*). The contribution of plasma In-111 to the time-activity curve recorded over the blood pool was estimated from a knowledge of the ratio of cell-bound to plasma In-111 activity in the peripheral blood samples; this was subtracted from the blood-pool time-activity curve (*2*). This ratio was never higher than 0.1, and usually less than 0.05. Plasma In-111 is not taken up appreciably by the spleen (*9*).

First pass. SBF, as a fraction of cardiac output (CO) was calculated as follows from the first-pass time-activity curve recorded over the spleen from 0 to 90 sec. This time-activity curve displayed a brief plateau (with count rate N_1) commencing about 30 sec after In-111 platelet reinjection (Fig. 1). Assuming that this plateau represents complete trapping of platelets entering the spleen on first pass before the arrival of recirculating platelets, then

$$N_1 = Q_i \alpha,$$

where Q_i is the quantity of In-111 platelets entering the spleen on first pass and α is a constant of proportionality.
If the equilibrium activity (i.e., at 30 min) in the spleen (Q_s) gives a count rate of N_2, then

$$N_2 = Q_s \alpha.$$

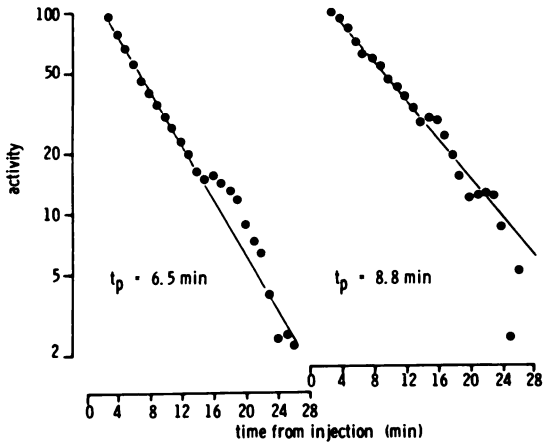

FIG. 2. Semilog splenic washout curves of In-111-labeled platelets predicted by deconvolution analysis. Ordinate: relative platelet activity; t_p = intrasplenic platelet transit time. Continuous lines are maximum-likelihood computer estimates for monoexponential function, which would be predicted by proposed model of platelet distribution. Left: normal subject; right: thrombocytopenic (20,000/μl) patient with reduced platelet life span (2 days) and splenomegaly (homologous In-111 platelets).

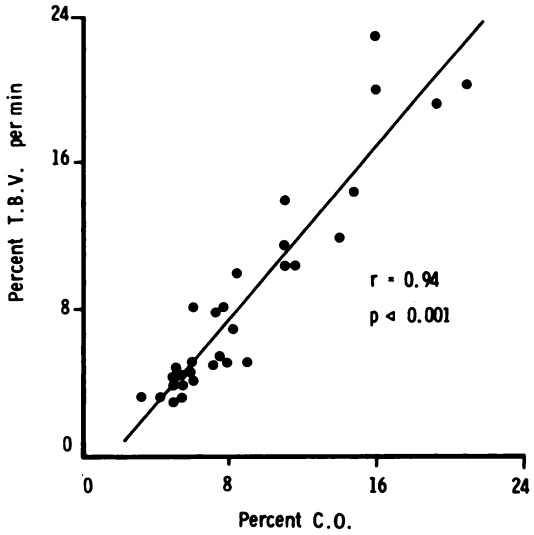

FIG. 3. Relationship between SBF calculated by compartmental analysis (percent TBV/min) and SBF calculated from first-pass splenic time-activity curve (percent CO). Continuous line = regression slope.

Hepatic activity at equilibrium (Q_h) was not quantitated but was assumed to be 12% of the dose (Q_T) (*8*).

$$\text{i.e., } Q_b + Q_s = Q_T - Q_h$$
$$= Q_T - 0.12\,Q_T$$
$$= 0.88\,Q_T.$$

Now

$$\frac{SBF}{CO} = \frac{Q_i}{Q_T}$$
$$= \frac{N_1}{Q_s + Q_b} \times \frac{0.88}{\alpha},$$

whence,

$$\frac{SBF}{CO} = \frac{Q_s N_1}{Q_b + Q_s} \times \frac{0.88}{\alpha} \times \frac{1}{Q_s}.$$

Then, substituting N_2/α for Q_s,

$$\frac{SBF}{CO} = \frac{Q_s}{Q_b + Q_s} \times \frac{N_1}{N_2} \times 0.88.$$

$\frac{Q_s}{Q_b}$ was measured as described under compartmental analysis (see above).

In subjects with a mean circulation time of 1 min, SBF based on this first-pass approach should be equal to SBF calculated from compartmental analysis.

Alternatively, since

$$\frac{SBF}{TBV} = K_s \frac{Q_s}{Q_s + Q_b},$$
$$0.88 \times \frac{N_1}{N_2} = K_s \times \frac{TBV}{CO} \qquad (4)$$

In other words, the ratio of the splenic count rates at completion of first pass and at equilibrium (or completion of uptake) should be very nearly equal to the rate constant of splenic uptake in subjects with a mean circulation time of 1 min

Intrasplenic platelet transit time. *Compartmental analysis.* Intrasplenic platelet transit time (t_p) was calculated from Eqs. 1 and 2.

Deconvolution analysis (*5*). This computerized technique makes use of the blood-pool and splenic time-activity curves to predict the time-activity curve that would be obtained over the spleen if the In-111 platelets were injected as a bolus directly into the splenic artery and did not recirculate. The curve so predicted was monoexponential (Fig. 2), and the rate constant (the reciprocal of t_p) was calculated. The assumption was made that the blood-pool time-activity recorded by the camera is an accurate reflection of the arterial In-111 platelet level.

RESULTS

At equilibrium, plasma In-111 was never higher than 10% of total blood In-111, and usually less than 5%. Mean platelet life span was quite variable in this heterogeneous group of patients, although in normal subjects labeled with this technique, it was 9.2–9.6 days.

Values of SBF, determined by compartmental analysis in this series, are given in the table, in which it can be seen that SBF is (a) raised in splenomegaly and (b) often raised (without splenomegaly) in diseases associated with elevated immune complexes. t_p did not correlate with any disease group or spleen size, but tended to fall with increasing splenic perfusion (i.e., SBF ÷ spleen volume), a relationship that we have previously documented (*2*, *10*).

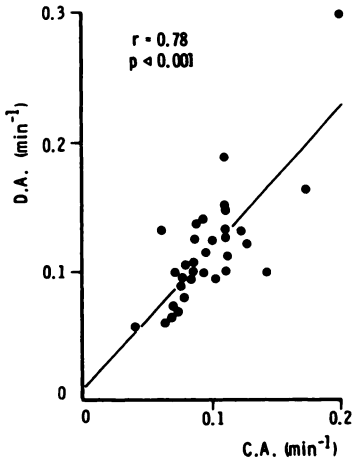

FIG. 4. Relationship between intrasplenic platelet transit time calculated from deconvolution analysis (DA) and that calculated from compartmental analysis (CA). Continuous line = regression slope.

There was a close correlation between SBF as a fraction of TBV per min and SBF as a fraction of cardiac output ($r = 0.94$, $p < 0.001$; Fig 3). The correlation between N_1/N_2 and $K_s/0.88$ was not quite as close ($r = 0.83$, $p < 0.001$). N_1/N_2 agreed approximately with $K_s/0.88$ (mean ratio 1.15 ± s.e.m. 0.05). The relationship between $1/t_p$, calculated from compartmental analysis and deconvolution analysis, is shown in Fig. 4. The correlation ($r = 0.78$) was highly significant ($p < 0.001$).

DISCUSSION

The main purpose of this study was to see whether SBF and t_p, when measured with In-111 platelets on the basis of compartmental analysis, were the same as when calculated by alternative techniques using different assumptions. Although the highly significant correlations tend to validate the analytical techniques used, it would be necessary to validate them using independently established methods. There are, however, no established methods of measuring either SBF or t_p in man, although our value for SBF in normal subjects (130–350 ml/min) is in good agreement with values obtained by others using more invasive techniques, e.g. Williams et al. (*11*), (100–300 ml/min). Validation of the technique in animals is subject to a number of difficulties, particularly interspecies variations in splenic structure and the response of the spleen to anesthetics and surgery.

Deconvolution analysis indicated that the distribution of platelet transit times through the spleen is consistent with a well-mixed, closed two-compartmental model, on which our method of measuring SBF is based. This, and the close correlation observed between t_p based on deconvolution and on compartmental analysis, serve as independent, indirect validation of the technique. Furthermore, the presence of In-111 platelet activity irreversibly trapped in the spleen as a result of cell damage during labeling is ruled out by the finding that the deconvolved splenic In-111 platelet clearance curves closely approached zero; i.e. did not reach an asymptotic value representing splenic In-111 platelet extraction.

There are a number of potential sources of error. Firstly, other sites of platelet pooling, in addition to the spleen, would create an apparent distribution volume in circulating blood greater than the true volume, thereby tending to underestimate SBF expressed as a fraction of TBV. Likely candidates for such sites are the lung (*12,13*) and liver (*14*). With respect to the lungs, a number of groups including ourselves (*8,15–17*) have found on quantitative imaging that soon after injection of In-111 platelets, the entire dose can be accounted for in the spleen, liver, and circulating blood, arguing against extrahepatosplenic sites of platelet pooling. Hepatic uptake of In-111 platelets, although less than the splenic (*8,15–17*), is completed very much earlier (between 2 and 6 min after injection) and remains essentially constant or falls slightly over the subsequent 30 min (*1,2,15*). The rapidity of equilibration over the liver suggests pooling, but the subsequent failure of hepatic activity to fall in parallel with blood-pool activity suggests irreversible uptake, presumably of cells damaged in the cell-labeling procedure. Secondly, on posterior imaging, the region of the cardiac pool would have recorded a substantial signal from the pulmonary blood pool. If, therefore, there was initial entrapment of platelet aggregates in the lung, the signal from the cardiac region, assumed to represent blood pool only, would effectively contain a background, with the result that the equilibrium count rate, as a fraction of the initial count rate, would be overestimated and Q_s underestimated. Firstly, however, platelet aggregates were not visible in the injectate, and secondly, excess lung activity is not supported by quantitative imaging (see above).

The clinial utility of SBF measurement in man is uncertain, and at present limited. However, interesting values of SBF are being recorded by us with this technique, particularly in patients with immune complex disease (*18*). The use of modified red-cell clearance as an indicator of splenic macrophage function, especially for the detection of reticuloendothelial blockade (*19–21*), is greatly enhanced by simultaneous measurement of SBF, since splenic extraction ratio, a clearly definable physiological parameter, can then be calculated (*22*). A noninvasive, reasonably accurate, method of measuring SBF in man would, therefore, clearly be of use. The technique also yields intrasplenic platelet transit time, although the clinical significance of this parameter is not known at present. Nonetheless, in the meantime the technique provides an experimental means of studying splenic blood-cell handling, an area in which there is relatively little information in spite of the widely recognized association between splenomegaly and

thrombocytopenia. An understanding of the mechanisms controlling intrasplenic platelet transit would provide a basis for therapeutic attempts to shorten t_p. Such a reduction in t_p, if it could be achieved without concomitant elevation of SBF, would raise the peripheral blood platelet count, particularly in patients with splenomegaly, in whom the size of the splenic platelet pool is increased.

ACKNOWLEDGMENTS

A.M.P. was supported by the Cancer Research Campaign.

Indium-111 was kindly supplied by Amersham International Ltd.

The authors are grateful to Miss Sally Barnes and Miss Beverley Shambrook for secretarial assistance.

APPENDIX

The interpretation of the splenic and blood-pool time-activity curves has been based on a two-compartmental model in which the input of indium is taken to be the product of splenic blood flow and arterial concentration, and the output proportional to the splenic indium content,

i.e., $$\text{blood} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} \text{spleen},$$

where K_1 is the fraction of TBV supplying the spleen per minute, and K_2 is the rate constant of clearance of platelets from the spleen (i.e., the reciprocal of mean transit time of platelets through the spleen). If Q_b and Q_s are the quantities of indium in blood and spleen, respectively, and

$$Q_b + Q_s = Q_o, \text{ the total extrahepatic indium,}$$

then

$$\frac{dQ_b}{dt} = K_2(Q_o - Q_b) - K_1Q_b,$$

and

$$\frac{dQ_s}{dt} = K_1Qb - K_2Q_s.$$

Integrating:

$$Q_b = Q_o\frac{K_2}{K_1 + K_2}[1 - e^{-(K_1+K_2)t}] + Q_oe^{-(K_1+K_2)t}$$

$$Q_s = Q_o\frac{K_1}{K_1 + K_2}[1 - e^{-(K_1+K_2)t}]$$

Thus, following injection of indium platelets, the blood indium will equilibrate with splenic indium, and they will both approach their equilibrium values at a monoexponential rate with a rate constant equal to the sum of K_1 and K_2. At equilibrium, K_2:K_1 will be equal to Q_b:Q_s, which can be measured from the time-activity curve for the blood pool, after correction for hepatic activity (*1*,*2*).

REFERENCES

1. PETERS AM, KLONIZAKIS I, LAVENDER JP, et al: Use of ^{111}indium-labelled platelets to measure spleen function. *Br J Haematol* 46:587-593, 1980
2. PETERS AM, LAVENDER JP: Factors controlling the intrasplenic transit of platelets. *Eur J Clin Invest* 12:191-195, 1982
3. ASTER RH: Pooling of platelets in the spleen: role in the pathogenesis of 'hypersplenic' thrombocytopenia. *J Clin Invest* 45:645-657, 1966
4. HARKER LA: The role of the spleen in thrombokinetics. *J Lab Clin Med* 77:247-253, 1971
5. WILLIAMS DL: Improvement in quantitative data analysis by numerical deconvolution techniques. *J Nucl Med* 20: 568-570, 1979
6. SINN H, SILVESTER DJ: Simplified cell labelling with indium-111 acetylacetone. *Br J Radiol* 52:758-759, 1979
7. DANPURE HJ, OSMAN S: Cell labelling and cell damage with indium-111 acetylacetone—an alternative to indium-111 oxine. *Br J Radiol* 54:597-601, 1981
8. KLONIZAKIS I, PETERS AM, FITZPATRICK ML, et al: Radionuclide distribution following injection of ^{111}In-labelled platelets. *Br J Haematol* 46:595-602, 1980
9. VIERAS F: Splenic uptake of ^{111}In. *J Nucl Med* 16:1205-1206, 1975
10. PETERS AM, LAVENDER JP: Platelet kinetics with indium-111 platelets: comparison with chromium-51 platelets. *Semin Thromb Hemost* 9:100-114, 1983
11. WILLIAMS R, CONDON RE, WILLIAMS HS, et al: Splenic blood flow in cirrhosis and portal hypertension. *Clin Sci* 34: 441-452, 1968
12. BIERMAN HR, KELLY KH, CORDES FL, et al: The release of leukocytes and platelets from the pulmonary circulation by epinephrine. *Blood* 7:683-692, 1952
13. MARTIN BA, DAHLBY R, NICHOLLS I, et al: Platelet sequestration in the lung with hemorrhagic shock and reinfusion in dogs. *J Appl Physiol* 50:1306-1312, 1981
14. HESSEL EA, SCHMER G, DILLARD DH: Platelet kinetics during deep hypothermia. *J Surg Res* 28:23-34, 1980
15. HEYNS A DU P, LÖTTER MG, BADENHORST PN, et al: Kinetics, distribution and sites of destruction of ^{111}indium-labelled human platelets. *Br J Haematol* 44:269-280, 1980
16. ROBERTSON JS, DAEWANJEE MK, BROWN ML, et al: Distribution and dosimetry of ^{111}In-labeled platelets. *Radiology* 140:169-176, 1981
17. SCHEFFEL U, TSAN M-F, MITCHELL TG, et al: Human platelets labelled with In-111-8-hydroxyquinoline: Kinetics, distribution and estimates of radiation dose. *J Nucl Med* 23:149-156, 1982
18. PETERS AM: Splenic blood flow measurement. In *Blood Flow Measurement in Man.* Mathie RT, ed. Castle House Publications Ltd, Tunbridge Wells, 1982, pp 227-239
19. FRANK MM, HAMBURGER MI, LAWLEY TJ, et al: Defective reticuloendothelial system Fc receptor function in systemic lupus erythrematosus. *N Eng J Med* 300:518-523, 1979
20. LOCKWOOD CM, WORLLEDGE S, NICHOLAS A, et al: Reversal of impaired splenic function in patients with nephritis or vasculitis (or both) by plasma exchange. *N Eng J Med* 300:524-530, 1979
21. WILLIAMS BD, PUSSELL BA, LOCKWOOD CM, et al: Defective reticuloendothelial system function in rheumatoid arthritis *Lancet* 1:1311-1314, 1979
22. PETERS AM, WALPORT MJ, ELKON KB, et al: Comparative clearance kinetics of heat damaged and antibody coated red cells. *Clin Sci* 62:7p, 1982