**111In-platelets dynamic study in chronic immune thrombocytopenic purpura**

Mila V. Todorović-Tirnanić¹, Vladimir B. Obradović¹, Smiljana V. Pavlović¹, Nada D. Suvajdžić², Ivo V. Elezović², Milica D. Čolović², Vladimir B. Bošnjaković¹

¹Nuclear Medicine Institute Clinical Centre of Serbia, Belgrade, Yugoslavia
²Haematology Institute, Clinical Centre of Serbia, Belgrade, Yugoslavia

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**Abstract**

BACKGROUND: The aim of this work was to estimate the significance of a dynamic study performed during the first 20 minutes after autologous 111In-oxinate-platelets injection in patients with chronic immune thrombocytopenic purpura (ITP). Two hypotheses were tested: a) dynamic study indicates the place of platelet sequestration; b) dynamic study reflects the quality of platelet separation and labelling procedure.

MATERIAL AND METHODS: Thirty-nine persons were investigated: 25 with shortened platelet life span (ITP), and 14 with normal platelet life span (6 healthy subjects and eight patients with myelodysplastic syndrome — MDS). Platelet blood count on the day of platelet labelling, general yield of platelet labelling (GYL), differential yield of platelet labelling (DYL), platelet life span, dynamic study with initial platelet accumulation in the liver (IPAL), sequential static study for determining the platelet sequestration index (SI) and platelet sequestration site (SS) were investigated.

RESULTS: Two types of labelled platelet kinetics were determined in both groups of patients: IPAL < 20% and IPAL > 20%. A statistically significant difference in GYL and DYL was noted between the patients with IPAL < 20% and IPAL > 20%. No significant difference was registered in platelet blood count, life span, SS and SI between the two groups of patients. Both yields of platelet labelling were higher in the group with IPAL < 20%. There was no correlation between IPAL and platelet SI, or between IPAL and platelet SS.

CONCLUSIONS: Dynamic study with 111In-platelets cannot predict platelet sequestration site in ITP patients, but it is a useful and sensitive method of platelet labelling procedure quality control.

Key words: labelled platelets, 111In-oxinate, platelet kinetics, chronic immune thrombocytopenic purpura (ITP), quality control of labelled cells

**Introduction**

The first initial platelet kinetics was performed in 1974. Radiouclide Tc-99m [1–4] was used for platelet labelling. The previously used chromium-51 in the form of sodium-chromate (Na₂CrO₄) enabled only the numeric data display, without visualisation of platelet kinetics capability, due to the low gamma photon yield (10%) during the radioactive decay of 51Cr, as well as to the inconvenience of the 51Cr gamma photon energy (320 keV) for gamma camera detection. Despite convenient gamma photon energy (140 keV) and yield of gamma photons (88.5%) during the radioactive decay of 99mTc, it could not enable late platelet kinetics investigation (2–9 days from the moment of labelled platelets intravenous injection), due to its short (6 h) radioactive half-life. This is the reason for non-acceptance of 99mTc as a radionuclide of choice for platelet labelling in thrombocytopenic patients, when platelet life span and sequestration site are needed.

Only two years later, in 1976, Thakur and collaborators [5] started using radionuclide 111In in the form of oxinate for platelet labelling. This enabled not only initial and late platelet kinetics investigation (2–9 days from the moment of labelled platelets intravenous injection), due to its short (6 h) radioactive half-life. This is the reason for non-acceptance of 99mTc as a radionuclide of choice for platelet labelling in thrombocytopenic patients, when platelet life span and sequestration site are needed.

The aim of this work is to estimate:

— the significance of dynamic study (initial labelled platelet kinetics), using 111In-oxinate labelled platelets in ITP patients (with...
shortened platelet life span) and in persons with normal platelet life span (control and MDS group).

Two hypotheses were tested:

- dynamic study indicates the place of platelet sequestration;
- dynamic study reflects the quality of platelet separation and labelling procedure;

- the influence of centrifugation speed on initial labelled platelet kinetics and on IPAL.

**Material and methods**

Dynamic study (initial labelled platelet kinetics) was performed in 39 persons: 25 ITP, 8 MDS and 6 control, healthy subjects (C).

ITP group consisted of 16 females aged 20–63 years (med = 33 yrs) and 9 males aged 13–71 years (med = 31 yrs). Platelet count in ITP group ranged from 17–110 × 10^9/l (med = 56 × 10^9/l). Ten patients were without therapy (Pt = 44–110 × 10^9/l, med = 67 × 10^9/l), and 15 ITP patients received small corticosteroid doses (Pt = 17–90 × 10^9/l, med = 42 × 10^9/l).

MDS group of patients consisted of two male persons aged 18 and 49 years (med = 33.5 yrs) and 6 women aged 35–62 years (med = 41.5 yrs). Platelet count on the day of labelling was 31–150 × 10^9/l (med = 59 × 10^9/l). Four patients were without therapy (Pt = 31–150 × 10^9/l, med = 47 × 10^9/l), while four patients were on small doses of corticosteroids (Pt = 55–85 × 10^9/l, med = 63 × 10^9/l).

The control group of healthy subjects consisted of four female and two male volunteers aged 31–43 years (med = 37.5 yrs). Platelet blood count ranged from 214–340 × 10^9/l (med = 263 × 10^9/l).

In vitro method of platelet labelling with 111In-oxinate was used. A blood sample was taken under ACD (anticoagulants volume participated with 20% in the blood sample volume). Three centrifugations, two slow (150 g) and one fast (900 g), were performed according to the method of Najeeb [13] in 20 platelet separation procedures. In 19 platelet separations the speed of fast centrifugation was lowered from 900 g to 716 g. Resuspended platelets were labelled with 10.4 MBq of 111In-oxinate. Non-bound radioactivity was lowered from 900 g to 716 g. Resuspended platelets were on small doses of corticosteroids (Pt = 55–85 × 10^9/l).

In order to determine DYL, two standards were made. A hundred microlitres of prepared platelet suspension was put into each of two (5 ml) tubes for gamma counter. 2 ml of saline solution was added to one of them (tube A), while in the other one (tube B) 2 ml of 1% ammonium-oxalate (in order to accomplish erythrocyte and leucocyte lysis) was added. Both tubes were centrifuged at 1500 g for 15 minutes. The supernatants were placed into two other tubes (A’ and B’). There were platelets, erythrocytes and leucocytes at the bottom of tube A. Platelet poor plasma (PPP) was the content of tube A’. At the bottom of tube B there were only platelets, while the content of tube B’ consisted of the lysed erythrocytes, leucocytes and PPP. Radioactivity of the four tubes (A, A’, B, B’) was measured in the gamma counter. Differential yield of platelet labelling Pt*/DYL was calculated using the formula: Pt*/DYL = B/(B + B’). Differential yield of PPP labelling was calculated according to the formula: PPP*/DYL = A’/(A + A’). Differential yield of E and Le labelling was calculated using the formula: (E*/ + Le *)DYL = 1 – (Pt*/DYL + PPP*/DYL).

After platelet labelling and standards preparing procedures (for DYL estimation), radioactivity of the syringe with labelled platelets was measured. At the time of the autologous labelled platelets intravenous injection, dynamic study (initial platelet kinetics investigation) lasting 20 min was initiated with the patient in PA position. During that time 40 frames of 30 s each were acquired in the matrix 64 × 64 in byte mode. Late platelet kinetics study was performed in all investigated persons in order to determine the index and the place of platelet sequestration [9]. Mean platelet life span was determined [6] too, as well as the number of injected labelled platelets [9].

When the dynamic study was accomplished, computer analysis of obtained data was performed using small, rectangular equal dimensions regions of interest (ROI) over the heart, the liver, the spleen and background (inside the patient, under the spleen but outside the kidney ROI). Time-radioactivity curves were obtained from all the ROIs. Background ROI radioactivity was subtracted from the liver and spleen ROI radioactivities. Initial platelet accumulation in the liver (IPAL) was calculated as a percentage of the liver radioactivity related to the summed liver and spleen radioactivity at 20th min.

The following statistical methods were applied in the analysis of obtained data: Fisher’s test of absolute probabilities, Wilcoxon-Mann-Whitney test of the range sums, and Student’s t-test.

**Results**

Two types of initial platelet kinetics were obtained: IPAL < 20% and IPAL > 20% in all investigated groups (Fig. 1, 2). In ITP group IPAL > 20% was obtained nine times, eight of which when fast (900 g) centrifugation during the platelet separation procedure was applied (Table 1). In MDS group IPAL > 20% was

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IPAL — initial platelet accumulation in the liver; ITP — chronic immune thrombocytopenic purpura; MDS — myelodysplastic syndrome; C — control group
obtained once, in the case when fast centrifugation was applied. In the control group IPAL > 20% was obtained twice, both times when fast centrifugation of blood samples was performed.

Fisher’s absolute probability test yielded significant difference in IPAL < 20% and IPAL > 20% frequency between the groups of patients whose blood samples were centrifuged under different centrifugation speeds (900 g or 716 g during third and fourth centrifugation) in ITP (Pe = 0.0076; Pt = 0.025; Pe > Pt; p < 0.05). Median IPAL value in ITP group with fast centrifugation was 21.3%, while in ITP group with slow centrifugation it was 12.9%. In ITP group under therapy arithmetic mean value for IPAL was 26.6% with fast centrifugation and median IPAL value with slow centrifugation was 13%. Fisher’s test did not yield a significant difference in the frequency of IPAL < 20% and IPAL > 20% between the groups with different centrifugation speeds for MDS and the control groups. IPAL values were 18.8% (for faster centrifugation speed) and 12.7% for lower speed in the control group.

Mean value (median) for platelet life span in ITP group with fast centrifugation was 1.5 days, while in the group with slow centrifugation it was also 1.5 days. Mean value (median) for platelet life span in MDS group of three patients with fast centrifugation of blood samples was 8 days, while it was 8.3 days in the group of five patients with slow centrifugation speed of blood samples. In four control subjects with fast centrifugation mean platelet life span was 8.9 days, while in two controls with slow centrifugation it was 9 days.

Median value for platelet blood count on the day of platelet labelling was 53 × 10^9/l in the group with faster (900 g) centrifugation, while it was 62 × 10^9/l in the group with slower centrifugation speed (716 g). Blood sample volume was 120 ml in the group with slower centrifugation and 71 ml in the group with faster centrifugation.

Platelet blood count in the control group was 273 × 10^9/l in the group with faster centrifugation and 270 × 10^9/l in the group with slower centrifugation. Sample volume was 50 ml in the group with faster speed and 25.5 ml in the group with slower centrifugation speed.

Wilcoxon-Mann-Whitney test yielded a statistically significant difference in the GYL values between the ITP group of patients with IPAL < 20% and IPAL > 20% (p = 0.028; p < 0.05). Median GYL value in the group with IPAL < 20% was 69.9%, and in the IPAL > 20% it was 39.9%. General yield of platelet labelling was higher in IPAL < 20%. Student’s t-test demonstrated a highly significant difference in GYL between the ITP patients (under therapy) with IPAL < 20% and IPAL > 20% (t = 8.207; p < 0.01). Arithmetic mean value for GYL in IPAL < 20% was 70.8%, and it was 42.4% in the group with IPAL > 20%. General yield of platelet labelling was higher in the ITP group under therapy with IPAL < 20%.

Student’s t-test yielded a highly significant difference in DYL between the ITP groups with IPAL < 20% and IPAL > 20% (t = 4.284; p = 0.003; p < 0.01). Arithmetic mean DYL value for the IPAL < 20% was 93.4%, and it was 77.6% in the IPAL > 20% group.
Differential yield of platelet labelling was higher in the IPAL < 20%. Platelet blood count mean value (median) in 16 ITP patients with IPAL < 20% was 63 × 10^9/L, and for 9 patients with IPAL > 20% it was 42 × 10^9/L. Patients under therapy had the values of 50 × 10^9/L and 38 × 10^9/L. Blood sample volume mean value for ITP patients with IPAL < 20% was 78.5 ml and for those with IPAL > 20% it was 120ml (76.5 ml for those without therapy and 120 ml for ITP patients under therapy). Platelet life span was 1.7 days in IPAL < 20%, and 1.4 days in IPAL > 20% (1 day and 1.2 days are the corresponding values for IPG group under therapy).

Mean value (median) for platelet sequestration index was 2.7 in the group with IPAL < 20%, and 1.1 in the group with IPAL > 20%. Patients with the highest values for IPAL (30.2% and 38.6%) had the sequestration index of 3.8 (spleen was the sequestration site) and 2.0 (sequestration was predominantly splenic). Wilcoxon-Mann-Whitney test showed that there was no significant difference in the life span (p = 0.317) or in sequestration index (p = 0.110) between the groups of patients with chronic ITP who had IPAL < 20% and IPAL > 20%. No correlation was found between IPAL on one side and platelet sequestration index or sequestration site on another (p = 0.021). Also, no correlation was found between IPAL and platelet life span (p = 0.086).

In MDS group of seven patients with IPAL < 20% mean platelet blood count value was 63.5 × 10^9/L, and in one patient with IPAL > 20% platelet blood count was 58 × 10^9/L. Mean blood sample volume was 80 ml in IPAL < 20% group, and 120 ml in IPAL > 20%. Mean platelet life span value was 8.4 days in IPAL < 20%, and 7 days in IPAL > 20%. Platelet sequestration index in IPAL < 20% group was 0.8, and it was 1.0 in IPAL > 20%. The patient with the lowest IPAL value (6.4%) had platelet sequestration index value of 0.7 (liver was the platelet sequestration site). General yield of platelet labelling (median value) for seven MDS patients with IPAL < 20% was 70.5% and for one patient with IPAL > 20% it was 23%. Differential yield of platelet labelling for patients with IPAL < 20% (median value) was 94.4% and for one patient with IPAL > 20% it was 67%.

Four controls with IPAL < 20% had platelet blood count mean value of 300 × 10^9/L, and two controls with IPAL > 20% had 258 × 10^9/L. Blood volume sample mean value in IPAL < 20% was 34.5 ml, and in IPAL > 20% 40 ml. Platelet life span in IPAL < 20% was 9.1 days and in IPAL > 20% it was 8.5 days. Platelet sequestration index in IPAL < 20% was 0.7, and it was 0.5 in IPAL > 20%. General yield of labelling in the control group with IPAL < 20% was 83.7%, and it was 84.9% in IPAL > 20%. Differential yield of labelling in IPAL < 20% was 98.6%, and it was 98.2% in IPAL > 20%. In two controls with IPAL > 20% problems during blood specimen collection (before platelet separation and labelling procedures) were encountered. Faster centrifugation protocol (900 g) was used thereafter. In the other two controls with fast centrifugation speed but without problems during blood specimen collection, IPAL was lower than 20%.

**Discussion**

In view of the different opinions concerning the significance of initial platelet kinetics investigation [8, 13], the aim of this work was to estimate its value. Can it:

— predict definitive platelet sequestration site, or

— indicate the quality of platelet labelling procedure?

Initial platelet kinetics was investigated during 20 minutes after autologous ^111^In-oxinate labelled platelets injection in 39 persons (25 ITP, 8 MDS and 6 controls).

Two different types of initial platelet kinetics were obtained in the liver and spleen in all investigated groups: ITP, MDS and control group. In the first type of initial platelet kinetics, the initial platelet accumulation in the liver (IPAL) was lower than 20% (Fig. 1). Time-activity curve obtained in the liver ROI was almost at the same level or lower than, the heart curve. This indicated that there was no temporary platelet sequestration, described as “collection injury” [13, 14]. Platelet separation and labelling procedure did not endanger the platelets. Their biodistribution was not changed [15]. Liver radioactivity reflected the presence of labelled platelets in its blood vessels [16].

In the second type of initial platelet kinetics, IPAL was higher than 20% (Fig. 2), time-activity curve from the liver ROI was continuously rising, overcoming the heart ROI curve. The liver curve level was higher compared to the heart curve till the end of initial kinetic study. This finding indicated that liver radioactivity overcame the radioactivity caused by the presence of labelled platelets in the blood vessels only. This was the result of platelet sequestration in the liver (“collection injury”) [13, 14, 17].

Our results indicate the presence of a significant difference in GYL and DYL between the groups of ITP patients with IPAL < 20% and IPAL > 20%. Both yields of labelling were significantly higher in the IPAL < 20% group. In the group with better-performed platelet separation and labelling procedure, we registered lower initial platelet accumulation in the liver during the first 20 minutes after autologous labelled platelets intravenous injection. This was obvious in our thrombocytopenic patients (ITP and MDS).

In control subjects (with normal platelet blood count) both yields of platelet labelling (GYL and DYL) were high, with almost no difference in obtained values in IPAL < 20% and IPAL > 20% group. We suppose that platelets in healthy subjects are less sensitive to slight injury than platelets in thrombocytopenic patients. Therefore we could not notice the difference in labelling yields between IPAL < 20% and IPAL > 20% group of control subjects. The reason for IPAL > 20% in two controls was probably a problem during the blood specimen collection, combined with the fast centrifugation (900 g) thereafter. In the other two controls with fast centrifugation speed but without problems during blood specimen collection, IPAL was lower than 20%. It seems that initial platelet kinetics reflects platelet derangement from the moment of blood specimen collection (for platelet separation and labelling procedure) till autologous labelled platelets intravenous injection.

Surprisingly, we noted a significant difference in the frequency of IPAL < 20% and IPAL > 20% between the ITP groups whose blood samples were centrifuged at higher (900 g) and lower (716 g) speeds. Initial platelet accumulation in the liver higher than 20% was registered in 8/13 ITP patients with fast centrifugation speed, and in 1/12 ITP patients with lower speed of centrifugation (Table 1). Higher speed of centrifugation led to a more frequent appearance of changed initial labelled platelet kinetics (IPAL > 20%). Nevertheless, the registered level of initial labelled platelet kinetics disturbance did not lead to a significant change in platelet life span in ITP, MDS and the control group. In subjects with normal platelet life span (MDS and control group), labelled platelet life...
on the late platelet kinetics. It did not indicate the definitive platelet sequestration site. Therefore it should be an integral part of each platelet separation and labelling. Initial 111In-platelet kinetics offers the possibility of the most sensitive labelled platelets quality control performance in each individual investigated person. Unchanged initial platelet kinetics (dynamic study), together with high GYL and DYL values, yields the desired level of diagnostic accuracy and reliability of the obtained results. Therefore it should be an integral part of each platelet sequestration site, life span and production estimation.

Initial platelet kinetics enabled us to optimise the method of platelet separation and labelling.

Conclusions

The results obtained indicate that initial 111In-platelet kinetics cannot predict definitive platelet sequestration site, which is determined by the late platelet kinetics.

Initial 111In-platelet kinetics reflects the quality of platelet labelling procedure. It is a more sensitive quality control parameter than platelet life span (in healthy subjects). It shows the quality of the whole procedure of platelet labelling, from the moment of taking the blood sample (for platelet separation and labelling) till the injection of 111In-platelets to the investigated person, while GYL and DYL reflect only a part of this procedure.

Initial 111In-platelet kinetics offers the possibility of the most sensitive labelled platelets quality control performance in each individual investigated person. Unchanged initial platelet kinetics (dynamic study), together with high GYL and DYL values, yields the desired level of diagnostic accuracy and reliability of the obtained results. Therefore it should be an integral part of each platelet sequestration site, life span and production estimation.

Initial platelet kinetics enabled us to optimise the method of platelet separation and labelling.

References