

COMPARISON OF PLATELET COUNTS IN SIMULTANEOUS VENOUS AND CAPILLARY BLOOD SAMPLES USING AN AUTOMATED PLATELET ANALYSER

D Y H Tai, K W Chan, Y C Chee, K H Mak

ABSTRACT

Platelet counts (PC) obtained simultaneously from capillary blood (CB) are generally lower than those from venous blood (VB)⁽¹⁾. We quantified this difference in 17 patients with low platelet counts (LPC) and 18 healthy volunteers with normal platelet counts (NPC). The reproducibility of the counts in these 2 groups of subjects was also evaluated.

The mean venous platelet count (VPC) and the mean capillary platelet count (CPC) were $67 \pm 30 \times 10^3/\text{ul}$ (\pm SD) and $61 \pm 23 \times 10^3/\text{ul}$ ($p = 0.012$) in the LPC, and $264 \pm 44 \times 10^3/\text{ul}$ and $234 \pm 45 \times 10^3/\text{ul}$ ($p = 0.00016$) in the NPC respectively.

The mean difference (d) in the PC between VB and CB were $9.4 \pm 13.1 \times 10^3/\text{ul}$ and $19.4 \pm 17.6 \times 10^3/\text{ul}$ in the LPC and NPC respectively.

The coefficients of variation (CV) of double counts for VB and CB were $8.1 \pm 8.3\%$ and $9.8 \pm 8.6\%$ for LPC, and $2.3 \pm 1.6\%$ and $2.5 \pm 2.2\%$ for NPC respectively.

In conclusion, VPC was frequently (82.9% or 29/35 cases) higher than CPC. In addition, in patients with LPC, the agreement between VPC and CPC was poor and the counts were less reproducible.

Keywords : platelet count, venous blood, capillary blood, reproducibility, dengue.

SINGAPORE MED J 1995; Vol 36: 263-266

INTRODUCTION

The platelet count (PC) is a common haematological parameter used in the management of patients in certain illnesses like dengue fever (DF) or dengue haemorrhagic fever (DHF). Decisions like when to discharge or transfuse patients are based strongly on the absolute PC and clinical parameters. Blood samples can be obtained by venepuncture - venous blood (VB), or by finger prick - capillary blood (CB). However, the physician may not be aware that the counts may differ between these two techniques. This factor may be important enough to give a significantly different PC and hence influence the decision-making of the doctor.

Brecher et al⁽¹⁾ laboriously investigated this difference using phase microscopy and manual counting on 13 normal subjects. They found that the mean CPC was significantly lower than VPC by 2.5% and the coefficient of variation (CV) of double counts for finger prick (17%) was about twice that for venepuncture (8%).

Studies comparing PC using automated machines are lacking. Therefore we conducted this study to evaluate and

quantify the difference in PC of simultaneous VB and CB samples in subjects with low platelet count (LPC) and normal platelet count (NPC). In addition, the reproducibility of results in these 2 groups of subjects was evaluated.

MATERIAL AND METHOD

Seventeen consecutive patients who were admitted with a clinical diagnosis of DF or DHF in November 1992 to the Department of General Medicine, Tan Tock Seng Hospital (TTSH) were recruited. All of these patients had VPC of $120 \times 10^3/\text{ul}$ or less and they formed the LPC group.

The NPC group comprised eighteen healthy volunteers from hospital staff and medical students. Their VPC were 180 to $385 \times 10^3/\text{ul}$.

The VB was obtained by venepuncture of the antecubital vein using a 3 or 5 ml Becton Dickson STTM slip tip syringe with a 19 or 20 SWG needle. A sample of 3 ml of VB was put into a plastic tube containing ethylenediamine-tetraacetic acid. This was performed by the respective house officers in the wards.

The CB was obtained by two delegated trained technicians with at least six years of relevant experience using the finger-prick method with an auto-click device. Similarly, the same technicians were delegated to prepare (dilute) the blood specimens for analysis by the automated analyser. This is to minimise inter-individual variation.

The VB and CB samples were obtained almost simultaneously within 15 minutes. They were then sent to the Clinical Laboratory, TTSH for analysis by the Baker 810TM Platelet Analyser. Analysis of the blood samples was done within half an hour to minimise changes in the platelet parameters with the passage of time⁽¹⁾.

All the blood films were screened by the technicians to avoid spurious readings due to platelet aggregation or abnormal platelets.

To determine the reproducibility of the results, each specimen of VB and CB was run twice.

The CV was used to measure the reproducibility of the analyser. This coefficient was derived from the equation : $CV = (\text{difference between runs 1 and 2 divided by the mean of$

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runs 1 and 2) x 100%.

The agreement between the two methods of counting platelets was assessed by the statistical approach proposed by Bland and Altman⁽²⁾.

The student's paired t-test was used to evaluate the differences. A p-value of less than 0.05 is considered as statistically significant.

The Baker 810™ Platelet Analyser identified and counted cells from 3 to 30 cubic microns as platelets.

Calibration was done by the maintenance engineer from the Baker Instruments Company once every 6 months using either a commercially prepared sample or whole blood. A 10-sample reproducibility was then carried out.

In addition, the Clinical Laboratory at TTSH has its own quality assurance programme to ensure precision and accuracy of results. Within-run and day-to-day reproducibility was performed on a commercially prepared sample, HAEM-PC (primary control) and whole blood patient samples (secondary control). The mean values, SD and CV were obtained and compared with the reference values in the product manual.

RESULTS

There were 10 female and 7 male patients in the LPC group. Their mean age was 30.4 ± 9.7 years with a range of 23 to 50 years.

The NPC group comprised 8 female and 10 male healthy subjects with a mean age of 28.4 ± 8.1 years and a range of 21 to 48 years.

The mean VPC was significantly higher than the mean CPC in both the LPC ($p = 0.012$) and NPC ($p = 0.00016$) groups (Table I).

The mean CV of the Baker 810™ Platelet Analyser used in this study is summarised in Table I.

The individual results of the CV of patients (LPC group) and normal subjects (NPC group) are illustrated in Fig 1.

The individual results of the greatest difference in PC between simultaneous VB and CB are shown in Fig 2. The greatest difference was the difference between the higher value in the VB minus the lower value in the CB or vice versa.

The mean (d) and SD (s) of the greatest difference in PC between simultaneous VB and CB in the LPC and NPC groups are summarised in Table II. The agreement between the two methods of counting the platelets is also reported (Table II).

We also found that the mean variation between VPC and CPC (using VPC as the denominator) was $14.0 \pm 19.6\%$ in the LPC group and $7.3 \pm 6.7\%$ in the NPC group.

DISCUSSION

We found that in 82.9% (29/35) of the cases, VPC were significantly higher than CPC in subjects with LPC and NPC using an automated platelet analyser. This observation was made by Brecher et al⁽¹⁾ about 4 decades ago in subjects with

NPC with manual counting. However the difference was higher (7.3%) using automated analyser compared to manual counting (2.5%) in persons with NPC.

Adhesion of a variable number of platelets at the site of the skin puncture and/or admixture of tissue fluid leading to very slight dilution of blood⁽¹⁾ may account for this finding. Ideally, large drops of blood which exude slowly but spontaneously should be used. However, gentle squeezing may be permissible. Otherwise, the results may be unreliable⁽³⁾. Another possible source of error is the presence of contaminating blood dried onto the outside of the capillary where it had been in contact with the flow. Such excess blood is difficult to wipe off without causing the loss of a portion of the blood contained within the capillary⁽⁴⁾.

Fig 1 - Reproducibility of platelet count results in low and normal platelet counts

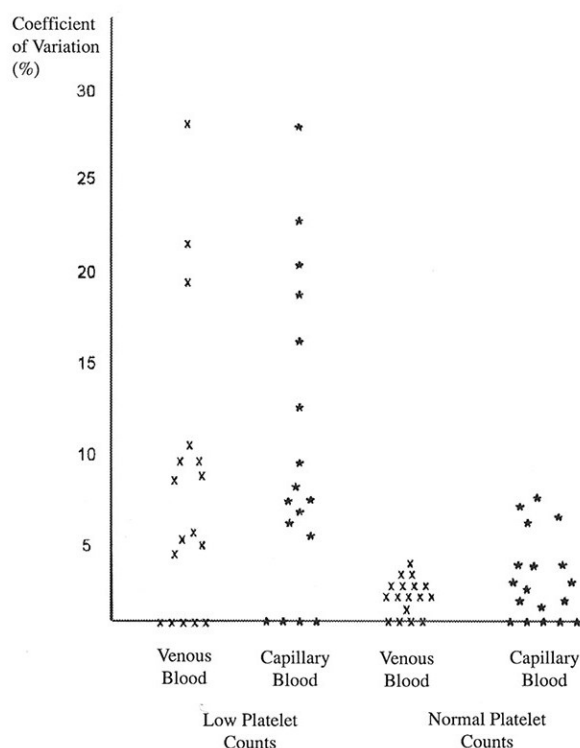


Table II - Difference in platelet counts (VPC - CPC) in subjects with LPC and NPC

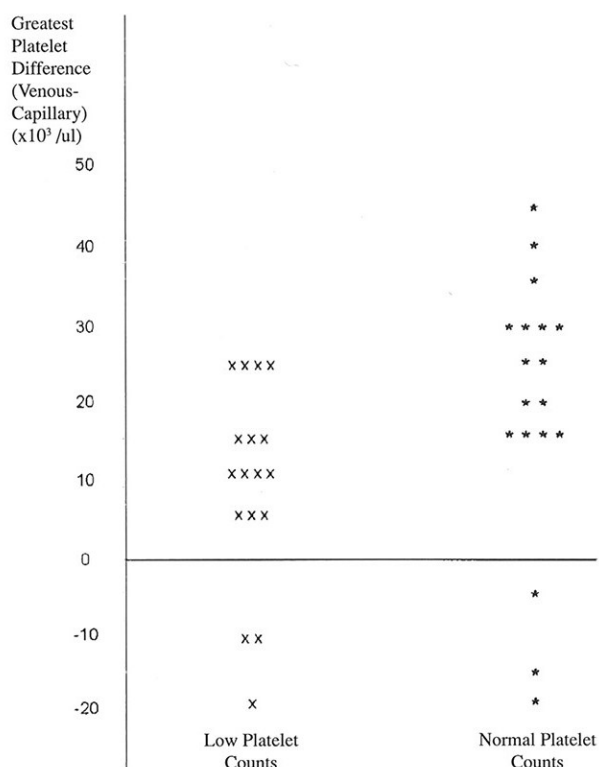
	LPC (n = 17) ($10^3/\text{ul}$)	NPC (n = 18) ($10^3/\text{ul}$)
Mean (d)	9.4	19.4
SD (s)	13.1	17.6
Range	-20 to 25	-20 to 45
Limits of agreement (d - 2s to d + 2s)	-16.8 to 35.6	-15.8 to 54.6
95% CI for the estimated limits of agreement (bias)	2.7 to 16.1	10.7 to 28.2
95% CI for the lower limits of agreement	-28.5 to -5.5	-30.9 to -0.6
95% CI for the upper limits of agreement	23.9 to 47.3	39.5 to 69.8

CI = confidence interval

Table I - Reproducibility of Baker 810™ Platelet Analyser

	LPC		NPC	
	VB	CB	VB	CB
Mean PC ($10^3/\text{ul}$)	67 ± 30	61 ± 23	264 ± 44	234 ± 45
Range ($10^3/\text{ul}$)	15 to 120	20 to 150	190 to 385	180 to 370
Mean CV (%)	8.1 ± 8.3	9.8 ± 8.6	2.3 ± 1.6	2.5 ± 2.2
Range (%)	0 to 28.6	0 to 28.6	0 to 4.4	0 to 6.3

Fig 2 - Greatest difference in platelet counts between simultaneous venous and capillary blood in low and normal platelet counts



Some commercially available methods⁽³⁻⁵⁾ have been developed to overcome this problem. They require less technical skill in drawing the correct amount of blood compared to the micropipette method. Hence, these methods are particularly suitable for use by the bedside or in the 'field'.

However, in 17.1% (6/35) of the cases in this study, it was observed that CPC were higher than VPC. This could be due to the variability of the automated machine and technical errors.

According to the Operator's Manual of the Baker 810TM Platelet Analyser⁽⁶⁾, the CV was higher in LPC compared to NPC and also in CB compared to VB. This might be due to the fact that the denominators used to calculate the CV were smaller in the LPC and CB respectively. Hence, in this study, we divided the subjects into those with LPC and NPC. Indeed, we found that the CV did not vary significantly from the values quoted in the Operator's Manual

A CV of 3 - 4% for normal range of PC is often quoted⁽⁷⁾. A normal range of PC is approximately 150 to 400 x 10³/ul with some studies reporting on upper limit of 500 x 10³/ul^(8,9).

The Singapore General Hospital and the National University Hospital used the Technicon H2 model. The quoted CV in the product manual for a normal PC of 385 x 10³/ul was 2.07% and the variability of the machine for LPC of 85 x 10³/ul was ± 15 x 10³/ul.

The CV of the Coulter STKS automated analyser used in Mount Elizabeth Hospital, Singapore for PC of 300 x 10³/ul, 30 x 10³/ul and 10 x 10³/ul were respectively less than 3.3%, 6.6% and 10.0%. Hence, the precision of the Baker 810TM Platelet Analyser used in this study is similar to that of the other models quoted above.

The Baker 810TM Platelet Analyser was the standard machine in the Tan Tock Seng Hospital, Toa Payoh Hospital

and Kandang Kerbau Hospital at the time of this study.

CPC deviated about 14.0% from the VPC in the LPC and only 7.3% in the NPC. This was because the absolute PC were lower in LPC compared to NPC. In absolute terms, the mean greatest difference in PC between simultaneous VB and CB was 9.4 ± 13.1 x 10³/ul with a range of -20 to 25 x 10³/ul in the LPC group. In subjects with NPC, the mean greatest difference in PC between simultaneous VP and CP was 19.4 ± 17.6 x 10³/ul with a range of -20 to 45 x 10³/ul. Hence, in patients with LPC, the agreement between VPC and CPC was poor and might pose difficulties in making clinical decision. When the PC were normal, the between-method differences were not important clinically as to affect medical judgement.

CONCLUSION

The reliability of automated platelet analyser may be affected by a number of factors such as the methods of obtaining the blood specimens (VB or CB), technical errors in the process of preparing (pipetting and diluting) the specimens for analysis, tendency of the platelets to aggregate, presence of extraneous particles in the diluent which may be mistaken for platelets, accuracy and precision of the automated platelet analyser⁽⁷⁾.

Physiological variation in the PC also has to be considered when interpreting results. There is variation during the course of a day as well as from day to day⁽¹⁰⁾. In some normal subjects there is a platelet cycle, with periods of oscillation of 21 - 35 days⁽¹¹⁾. A fall in PC may occur in normal women about the time of menstruation⁽¹²⁾.

In interpreting PC, we should take the above into consideration and not act on isolated out-of-the-way reading. A single abnormal PC should always be confirmed by a second count and inspection of the blood film. This is especially important in patients with LPC. Because of the poor agreement between VPC and CPC in thrombocytopenic patients, the "true" value should be verified by a VB sample before making important clinical decision such as platelet transfusion.

ACKNOWLEDGEMENTS

I wish to record my thanks to the following who had contributed to make this paper possible:

- 1) Mr Cheang Fook Weng, Chief Medical Technician and Mr Yeo Chiang Guan, Medical Technologist, Clinical Laboratory, TTSH for their excellent collaboration in the study.
- 2) Nurses of Wards 54 and 2, TTSH and 3rd year medical students who were posted to the Department of General Medicine, TTSH in Nov 1992 for volunteering themselves as normal subjects.
- 3) Miss Ngui Sieh Fah for secretarial assistance.
- 4) Miss Lim Meng Tsui, Information and Service Section, TTSH and Mr Timothy Ng for helping in the data analysis and generating the graphs.
- 5) Mr Rajaram s/o Sivalingam, Laboratory Technician, TTSH for doing the finger prick on patients and normal subjects and lending me some of his reference books.

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