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Vascular Pharmacology 39 (2002) 123–125

Vascular Pharmacology

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Blood sample stability at room temperature for counting red and white blood cells and platelets

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Received 1 August 2002; accepted 1 August 2002

Abstract

Blood handling required for different cellular variables is different. In a practical setting of blood sampling approximately 4 h separated from the first analysis, we compared the analysis of blood cell variables at this 4-h point with analysis of blood stored for ~48 h (over the weekend) at room temperature. Blood was collected from 304 apparently healthy individuals aged between 17 and 70 years, with a female/male ratio of 1.8, in K_3EDTA. Measurement was performed with a Beckman Coulter Counter Maxm. In addition to the comparison of the data and their correlation on the two time points, we investigated agreement between the data using analysis according to Bland and Altman. Counts of white and red blood cells and platelets were found stable over time and agreement of data was excellent. Platelet mean volume increased as expected between the two time points from 8.8 to 10.3 fl. The white blood cell subpopulations, however, changed over time with a decrease in neutrophils and monocytes and increases in lymphocytes and eosinophils. Apparently, ageing of the sample resulted in the alteration of certain cell characteristics leading to a change in automated cell classification without changing the total number of cells. Among the preanalytical variables recorded, only the time of the year and gender were found to be minor determinants ($r < .25$) of some of the differences between ~4 and ~48 h analysis delay. It is concluded that after storage at room temperature over approximately 48 h counts of red, total white cells, platelets and analysis of platelet volume can be combined in one assay session.

Keywords: Blood; Platelets; Red and white blood cells

1. Introduction

Both white blood cell count and mean platelet volume are variables of interest in epidemiological and clinical studies in view of their relation to inflammation and role of risk markers for cardiovascular disease (Yarnell et al., 1991; Ernst et al., 1987; Martin et al., 1991).

The change in platelet volume with time after blood sampling provides a practical problem when analysis has to be postponed (Lippi et al., 1987). For the evaluation of mean platelet volume, it has been proposed to wait for >48 h with analysis to allow for the swelling in EDTA blood to be completed (Martin et al., 1991). The volume of the swollen platelet has been shown to be proportional to the original volume (Martin et al., 1991).

In a practical setting of blood sampling of twins in weekend sessions, we evaluated whether blood cell analysis could all be performed after 48 h at Monday after the weekend while blood was kept at room temperature.

In a set of 295 individuals, we analysed K_3EDTA-blood on the day of the sampling within 4 h and 48 h later after storage at room temperature.

2. Methods and individuals

EDTA blood was collected from 304 apparently healthy individuals aged between 17 and 70 years, with a female/male ratio of 1.8.

Measurement was performed with a Beckman Coulter Counter Maxm. delivering a standard package of variables including counts of red blood, white cells and platelets, and percentages of neutrophils, lymphocytes, monocytes, eosinophils, basophils and mean platelet volume.

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1537-1891/02/$ – see front matter
PII: S1537-1891(02)00298-7

Several preanalytical variables (age, gender, medication, use of oral contraceptives, fasting, smoking and pregnancy) were recorded.

In addition to the comparison of the data and their correlation on the two time points, we investigated agreement between the data using analysis according to Bland and Altman (1995).

3. Results

From the 304 individuals sampled, 295 adequate pairs of analysis could be evaluated. Table 1 shows the mean or median values at first and second analyses, Spearman's correlation coefficient and the presence of agreement.

As shown in Table 1, correlation between data on the 2 days and agreement was excellent for counts of white and red cells and platelets. White blood cell count showed the best correlation.

Considering the paired analysis as duplicates, the calculated coefficient of variation (CV) was excellent for all three variables showing for white blood cell count a CV (median) of 1.2% and an interquartile range of 0–1.8%; for red blood cell count a CV (median) of 0.8% and an interquartile range of 0.3–1.3%; for platelet count a CV (median) of 2.8% and an interquartile range of 1.1–4.6%.

For the pairs, the calculated CV of red blood cell counts showed a bimodal distribution (below and above 4%). Twenty pairs showed CV >4%. Before removing these 20 pairs, there was a correlation between the CVs of platelets, white blood cells and red blood cells. After excluding these 20 pairs, this correlation was not present anymore.

The white blood cell subpopulations showed poor correlation between the two time points and generally no agreement. The detection of subpopulations apparently changed over time with a decrease in neutrophils and monocytes and an increase in lymphocytes and eosinophils. Fig. 1 shows the apparent changes on the percentage of monocytes for 15 representative samples.

Among the preanalytical variables recorded, only the time of the year and gender were found to be minor determinants ($r<.25$) of some of these differences between fresh and 48 h samples.

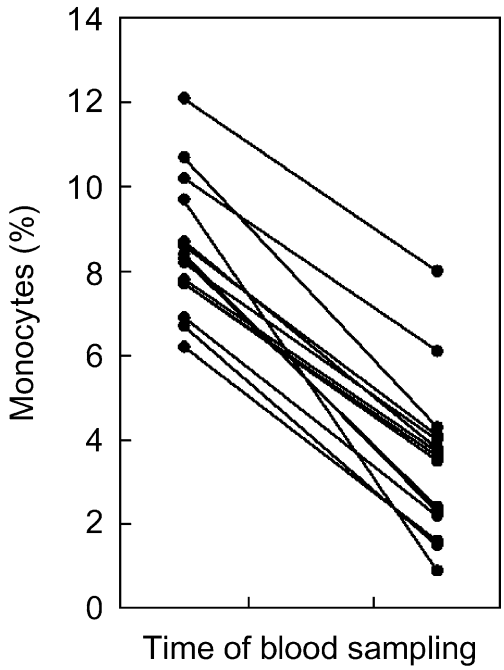

Fig. 1. Effect of time between blood sampling and laboratory processing on the percentage of monocytes.

The mean platelet volume increased in time and volumes before and after incubation showed an excellent correlation (R=.920; Spearman's). The mean platelet volume increased from 8.8 to 10.3 fl with a significant individual variability (S.D. of the increase of 17.7% is 4.8%.

The increase is only to a very limited extent dependent upon the initial platelet volume (r=−.142; P=.014).

4. Conclusion

Apparently, ageing of the sample at room temperature for ∼48 h after the initial ∼4 h did not result in changes of counts of red blood cells, total white cells and platelets. In 20 samples, the change in red blood cell count was larger (>4%), indicating in ∼7% of the samples an instability which was predominantly expressed in red blood cell count and least in white blood cell count.

Table 1
Mean or median values at first and second analyses, Spearman's correlation coefficient and the presence of agreement

Cell type	Mean or median[a] for 'fresh' analysis	Mean or median[a] for analysis after 48 h	Spearman's correlation coefficient	Agreement according to Bland and Altman
RBC ($\times 10^{12}$/l)	4.58	4.62	.95	Yes
WBC ($\times 10^{9}$/l)	6.05[a]	6.00[a]	.99	Yes
Platelets ($\times 10^{9}$/l)	242	238	.94	Yes
Neutrophils (%)	57.1	54.3	.76	No
Lymphocytes (%)	31.5	36.8	.70	No
Monocytes (%)	8.31	4.50[a]	.48	No
Eosinophils (%)	2.15[a]	2.70[a]	.79	Yes
Basophils (%)	0.20[a]	0.20[a]	.27	No

[a] Median.

Within the population of white cells, the incubation at room temperature resulted in the alteration of certain cell characteristics leading to a change in automated cell classification in the device used without changing the total number of cells. It can be concluded that this differential classification cannot be done on samples stored at room temperature.

Also, for the platelet volume, storage resulted in changes, but these were intended to take place. We did not check whether or not the swelling was completed in 48 h, but we observed a good relation between ''fresh''(∼4 h) and delayed analysis (∼48 h).

It can be concluded that for the variables of white blood cell count, platelet count and platelet volume, the analysis in blood stored for 48 h at room temperature can be used, however, for specific analysis of white blood cell differentials, fresh analysis or refrigerated storage remains the only approach.

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