The effect of Pre-analytical factors on circulating monocyte subpopulations

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Abstract

The aim of this study is to see if various pre-analytical factors affect the distribution of monocyte subpopulations. The pre-analytical phase is the most error prone at biomedical clinical laboratories and may have consequences for patients, from wrong diagnosis to un-necessary retesting.

Circulating monocytes can be divided into three subset based on expression of CD14 and CD16 surface markers. $CD14^{Bright}CD16^{negativ}$ monocytes are considered classical monocytes, $CD14^{Bright}CD16^{dim}$ are intermediate monocytes and $CD14^{Dim}CD16^{bright}$ are nonclassical monocytes.

The chosen pre-analytical factors was the choice of anticoagulant in the test tube, elevated concentration of anticoagulant in the test tube (underfilling of test tubes), storage conditions and freezing and thawing. Wrong test tube (with a less optimal anticoagulant) and not completely filled test tubes are commonly reported pre-analytical errors. The latter two was chosen based on their significance. For example, blood sampling procedure is sometime preformed outside of the laboratory and samples are sent to the laboratory. Freezing cells is a well establish method at almost all laboratories working with cells. The aim is to inspect if any of this affect circulating monocytes.

The study is in its final phase, and we present here some preliminary results

Methods

Anticoagulant: 22 donors, predominately young females, volunteered to the study. A blood sample of ACD-a, Li-Heparin and K_3 was drawn from each participant. For the Heparin and EDTA tubes, differentiated cell count was preformed on ABX pentra XL80.

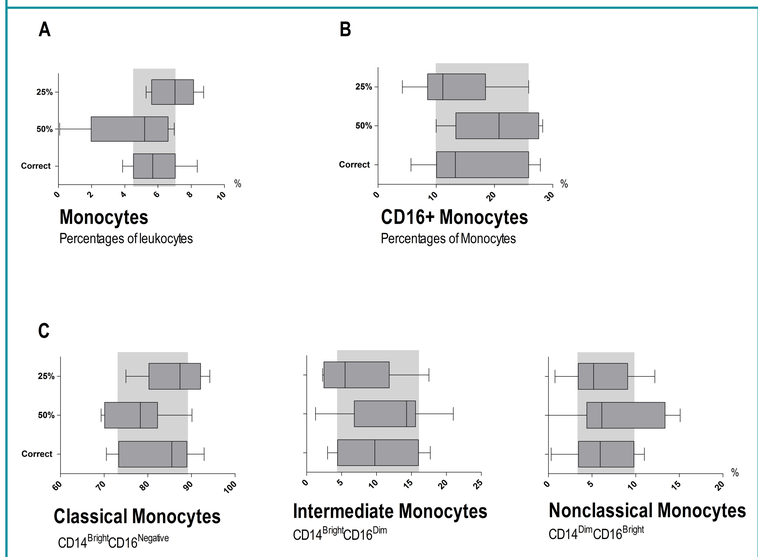
Underfilling of test tubes: The staff of the blood bank at Haukeland university hospital randomly chose 8 donors. Blood was collect in ACD tubes. For each donor three tubes was collected. One tube was correct filled, one was filled halfway (50%) and one was approximately filled one fourth (25%).

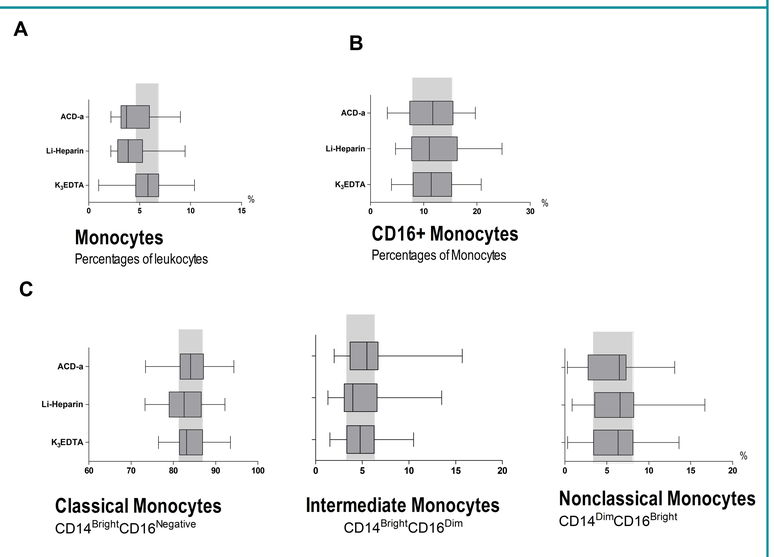
Flow cytometry and statistics: Whole blood was stained with antibodies before the sample was lysed. The sample was run on a BD FACSverse flow cytometer and the data was analysed by FlowJo vX. Statistical analysis was preformed in IBM SPSS statistics 23 (One Way Anova test, p>0,05 was considered significant)

Results

No significant difference was found between the different filled tubes, except for the percent monocytes of leukocytes (p= 0,033). The test tubes with the highest concentration of anticoagulant (filled 25 %) gave the highest percentages of monocytes. There was also found a significant difference in the total percentage of monocytes between the various test tubes (p = 0,022), with EDTA tubes giving the highest percent of monocytes. For the distribution of monocyte subsets, no significant difference was found.

From the differentiated cell count data, the following parameters was significant difference between EDTA and heparin blood; haemoglobin (HGB), platelets (PLT), Plateletcrit (PCT), Platelet distribution with (PDW), large immature cells (LIC), Eosinophils (EOS), monocytes (MON) and Lymphocytes (LYM) (Data not shown).





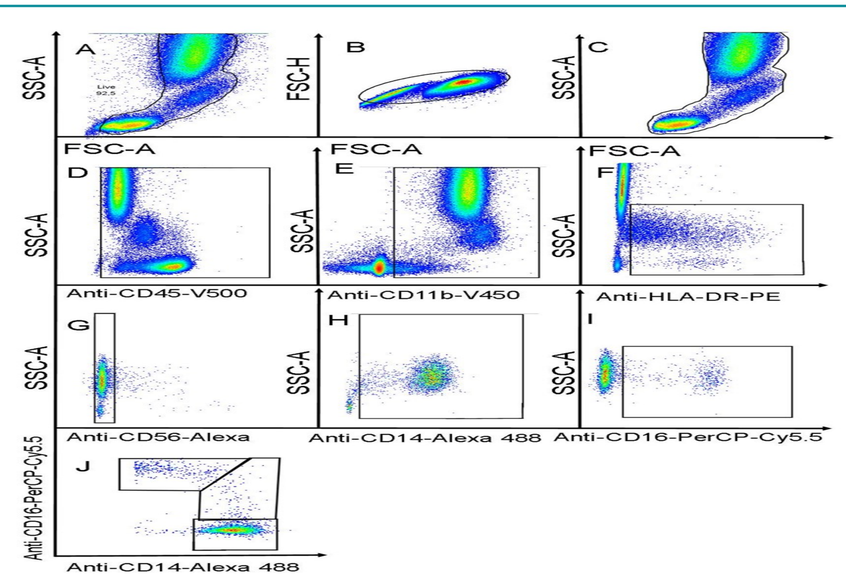


Figure 1) Gating strategy A) Live cells were gated to exclude debris/dead cells based on SSC-A and FSS-A properties. **B)** Live cells where plotted FSC-H vs FSC-A and gated around single cells. From the singlet gate a new **C)** SSC-A/FSC-A dot plot and a gate of Total Cells. In the Total Cells gate, SSC-A vs CD45 were plotted, and CD45+ events gated **D)** The CD45+ cells were plotted SSC-A vs CD11b **E)** and the CD11b+ cells were plotted SSC vs HLA-DR **F).** $CD45^{+}CD11^{+}HLA\text{-}DR^{+}$ cells were plotted SSC-A vs CD56 A647 and the CD56- cells was gated **G).** $CD45^{+}CD11^{+}HLA\text{-}DR^{+}$ $CD56^{-}$ was plotted SSC-A vs CD14 **H).** The CD14+ cells were considered Monocytes. From the Monocyte gate, SSC-A vs CD16 plot and CD16+ cells were gated **I).** Again in the Monocyte gate, **J)** CD16 were plotted against CD14 and gates are made around Classical (CD14++CD16-) monocytes, Intermediate (CD4++ CD16+) and Nonclassical (CD14+CD16++) monocytes.

Figure2) Underfilling of ACD-a test tubes A) Percent monocytes of leukocytes in the test tubes filled differently. Median; 7,2% (25 % filled), 5,2% (50 % filled) and 5,2 % (correct filled) **B)** Percent CD16+ monocytes in the different filled test tubes. Median 12 % (25 % filled), 24,5% (50 % filled) and 14,5 % (correct filled) **C)** distribution of monocyte subpopulation; classical monocytes, intermediate monocytes and nonclassical monocytes. Median classical monocytes 85,7 % (25 % filled), 75,2 % (50 % filled) and 82,7 % (correct filled). Median intermediate monocytes 5,6 % (25 % filled), 14,8 (50 % filled) and 12,6 % (correct filled). Median nonclassical monocytes 5,1 % (25 % filled), 5 % (50 % filled) and 4,7 % (correct filled).

Figure 3) Anticoagulant A) percentage of monocytes of total leukcytes. Median; 3,7 % (ACD), 4,3 % (Heparin) and 5,8 % (EDTA). **B)** Percent CD16+ Monocytes. Median; 11,7 % (ACD),11,1 % (Heparin) and 11,4 % (EDTA). **C)** Percentage of the various monocyte subpopulations; Classical Monocytes, Intermediate Monocytes and Nonclassical Monocytes. Median of Classical Monocytes (ACD) 84,1%, 82,6 (Heparin) and 83,1% (EDTA). Median of Intermediate Monocytes; 5,5 % (ACD), 4,0 % (Heparin) and 4,8 % (EDTA) Median of Nonclassical Monocytes; 6,5 % (ACD), 6,6 % (Heparin) and 6,4 % (EDTA).

Conclusive remarks

The choice anticoagulant did not have an impact on the distribution of monocytes, it did have a impact on the total percentage of monocytes. Similar result was observed when analysing test tubes filled to various grades.

The likely explanation for this is activation and aggregation of platelets and attachment of monocytes to the test tube surface. The variation between EDTA and Heparin blood in platelet (PLT), plateletcrit (PCT) and platelet distribution from cell count show that Heparin activates platelet aggregation, reported in coherence with other studies. For the ACD blood, the lower percent monocytes might be explained by adherence of monocytes to the test tube. No data on the actual cell numbers was obtained for the ACD-a tubes, which should perhaps been done.

For the underfilling experiments, one explanation could be the large size of the test tubes. The correct filled test tubes contains approximately 8 ml of blood, so the least filled tubes contains approximately 2 ml of blood. Other studies have shown that only at lower volumes was the parameters affected. Smaller volumes, such as 1 ml or 0,5 ml could possibly have an impact on the distribution of monocyte subsets. Similar experiments with EDTA blood is planned, then smaller tube will be used to investigate this.

Future perspectives

Storage conditions, sample stored at either room temperature or cold for up to 72 hours. Samples will be analysed immediately, the day after (18-24 hours) and after 72 hours. PBMCs have been separated by density gradient separation and frozen in nitrogen tanks. Applied is the protocol used at the haematology research laboratory at Haukeland university hospital. Currently, all the frozen PBMCs are from ACD-a blood samples and EDTA blood will be included. Cells will be stained with CD14 and CD16 markers along with a live/dead marker. Test of undefiling of EDTA test tubes is planed.

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