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Determination of the Residual White Cells in Blood Components. Experience of Tor Vergata Transfusion Service

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Introduction

The transfusion of blood components is associated to the risk of adverse events associated to alloimmunization against HLA-antigens or Graft versus Host Disease (GvHD) and haematological patients are often involved because of the high transfusion support. To limit this risk, a reduction of residual WBCs in blood components is recommended by European guidelines and an accurate enumeration of residual WBCs in leukoreduced products is mandatory for Transfusion Services.[2]

WBCs are counted by automatic analysers, routinely. They are able to detect blood cell number and size: the cells pass through a small hole, where electrical current is applied. The greater is the cell, the greater is the increase of impedance.

Some centres count them by flow cytometry. This technique allows an automatic analysis of cellular suspensions and an evaluation of physical properties of cells contained in a laminar flow, using fluorochrome conjugated monoclonal antibodies. Many parameters are observed and recorded: volume, presence of granules, fluorescence.

Our goal is to ensure the efficiency of our production process and we used both automatic analyser and flow cytometer for accurate counting of residual WBCs in blood components. Our partnership to UK NEQAS program (Low Level Leucocyte Counting Trail), organised by the Sheffield University Hospital, guarantees a high level of accuracy.

Materials and Methods

900 samples of blood components have been evaluated from September 2015 to December 2016 at the Transfusion Service of Tor Vergata which is hub for the others. They were produced by Tor Vergata (160), Velletri (480), Frosinone (220) and Campus Biomedico Transfusion Services (40) (Table 1).

Platelet and RBC samples were evaluated within 24 and 72 hours from the whole blood separation respectively. The volume of different concentrates is reported in Table 2.

1 ml sample from PLT or RBC concentrates was obtained and then processed with Sysmex 1800 automatic analyser and BD FACScanto II cytometer. The cell count is obtained employ-

Table 1. Transfusion Services enrolled and blood components tested

	Red cell concentrates	Platelet concentrates
Tor Vergata	80	80
Velletri	240	240
Frosinone	110	110
Campus Biomedico	20	20

Table 2. Volume of RBC and PLT Concentrates (ml)

Transfusion Services	RBC concentrates (ml)	PLT Concentrates (ml)
Tor Vergata	272 (239-312)	63 (27-78)
Velletri	272 (192-325)	63 (41-109)
Frosinone	275 (180-304)	62 (45-102)
Campus Biomedico	275 (262-302)	61 (44-97)

Data are shown as median values and range

ing electrical current: the greater is the cell, the greater is the increase of impedance. Cytometry is a very simple, quick and precise technique. The preparation of the sample is the most delicate phase in cytometry: 0,1 ml sample is dispensed into a Trucount tube, in which a lyophilised pellet is present with a predefined number of counting beads. A 400 µl BD Leucocount Reagent, containing RNAse, is added: this enzyme digests RNA. It contains also a detergent which makes permeable the cell membrane, allowing the Propidium Iodide (PI) to stain cellular DNA. Then, there is an incubation time of ten minutes at room temperature.

At the end, acquisition and analysis with the flow cytometer takes place.

Results

Cytometer is almost ten times more sensible than the automatic counter in detecting WBCs in RBC concentrates as shown in Table 3.

Cytometer and analyser give similar results in PLT concentrates. The sensibility of the two techniques is very similar. Complete results are shown in Table 4.

Discussion

Our institution is a reference point for the treatment of haematological neoplastic diseases and many patients receive transfusion therapy daily.

It is important to avoid transfusion reactions that are very common in these patients. First of all, the occurrence of alloantibodies against leukocyte antigens can cause a Non Haemolityc Transfusion Reaction (NHTR), characterized by fever and widespread malaise.[3,4,6] By contrast, leukoreduced blood components rarely cause NHTRs.

Residual white cells can also induce GvHD-Transfusion Associated (GvHD-TA).[1] This is a very serious complication: it happens when patients are immuno-depressed, because of the allogeneic T-lymphocytes attack to recipient's tissues.

Nowadays GvHD-TA does not yet occur, because of the irradiation practice of cellular blood components in such patients.

All these complications may lead to a longer hospitalisation, with an increased risk of hospital infections, the need of corticosteroid and antibiotics administration, a worse quality of life and an increase of public health costs.

In order to avoid these complications, the recommendation n R (95) 15 contains the current regulations on preparation, use and quality characteristics of blood products and a strict control of the residual white cells contained in blood products is required.[2,7] The recommendation is applied in Tor Vergata Service, not only in the blood units collected in the hub, but also in those collected in the other Services.

Our experience shows that there is a high correlation between the data obtained by Sysmex 1800 and Cytometer BD-Canto II for PLT concentrates (Table 4). A very small number of blood components from Campus Biomedico has been assessed up till now and this is the reason why data are not shown. On the contrary, the correlation between the two techniques is very low in erythrocytes concentrates (Table 3).

Conclusion

It is very important for Tor Vergata Transfusion Service to guarantee for the patients a safe transfusion therapy in order to improve patient's quality of life, reduce the hospitalisation time and care costs, avoid the administration of drugs after transfusions.

We have shown that common analysers are able adequately to count the number of white blood cells that are contained in platelet concentrates. Thus, cytometry is not recommended to count WBCs in platelet concentrates. However, the use of analysers is not satisfactory in counting WBCs in erythrocytes concentrates, because the sensibility of this technique is very low in this setting. So the introduction of cytometry is a better technique to assess the quality of our erythrocyte products.[8]

Table 3. Residual WBCs/µl in RBC concentrates counted by SYSMEX 1800 and BD FACS CANTO II

Transfusion Services	Sysmex 1800 (wbc/µl)	BD Facs Canto II (wbc/µl)
Tor Vergata	10 (0-80)	100 (0-1900)
Velletri	0 (0-100)	110 (0-1500)
Frosinone	0 (0-70)	110 (0-700)
Campus Biomedico	0 (0-10)	100 (0-500)

Data are shown as median values and range

Table 4. Residual WBCs/µl in PLT concentrates counted by analyser Sysmex 1800 and cytometer BD FACScanto II

Transfusion Services	SYSMEX 1800 (wbc/µl)	BD FACS CANTO II (wbc/µl)
Tor Vergata	400 (80-2600)	400 (20-4500)
Velletri	390 (20-4600)	380 (1400-3900)
Frosinone	400 (30-2800)	400 (160-2800)
Campus	280 (30-1200)	210 (300-1300)

Data are shown as median values and range

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