Transfusion Medicine and Hemotherapy

Review Article · Übersichtsarbeit

Transfus Med Hemother 2004;31:152–161

Received: April 6, 2004
Accepted: April 22, 2004

Flow Cytometry in Transfusion Medicine: Development, Strategies and Applications

B. Greve[a] G. Valet[b] A. Humpe[c] T. Tonn[d] U. Cassens[e]

[a] Institut für Strahlenbiologie, Universitätsklinikum Münster
[b] Max-Planck-Institut für Biochemie, Martinsried
[c] Dr. Mildred-Scheel-Haus der II. Medizinischen Klinik und Poliklinik des Universitätsklinikums Schleswig-Holstein, Campus Kiel
[d] DRK-Blutspendedienst Baden-Württemberg-Hessen, Institut für Transfusionsmedizin und Immunhämatologie Frankfurt am Main
[e] Institut für Transfusionsmedizin, Universitätsklinikum Münster, Germany

Key Words
Flow cytometry · Transfusion medicine · Applications

Summary
Nowadays, flow cytometry represents an essential tool for the daily work in laboratories for transfusion medicine. After cytophotometry of immobilized cells, flow cytometry was developed since the 1960s using moving fluorescence-stained cells. This technique enabled the determination of several thousand cells per second, e.g. from blood, bone marrow, or organ-associated cell suspensions. The introduction of monoclonal fluorescence-labeled antibodies in diagnostics has further accelerated the development of flow cytometry. In the meantime, numerous cellular and nuclear properties can simultaneously be measured on single cell level in automated devices at high speed and precision. Internal standards and quality control systems further increased the accuracy of flow cytometric analyses. Flow cytometry is therefore likewise suitable for routine diagnostics in patients, quality control of blood components and scientific purposes in transfusion medicine. Nevertheless, many flow cytometric applications are related to counting of rare events or detection of cells with low antigen expression. Therefore, several pre-analytic and analytic factors (e.g. erythrocyte lysing procedures, cell gating strategies, etc.) may essentially affect the accurate determination of cell numbers and functions. In this issue, TRANSFUSION MEDICINE AND HEMOTHERAPY starts a recurrent sequence of reviews focused on 'Flow Cytometry in Transfusion Medicine'. In order to introduce the flow cytometry series, this review summarizes the development, principles, and strategies of flow cytometry as an increasing diagnostic tool in medical laboratories. Also, current and possibly future flow cytometric applications in transfusion medicine are epitomized, e.g. quality control of blood products, immunohematologic diagnostics, hematopoietic progenitor cell transplantation, adoptive immunotherapy, and further therapeutic applications. In following issues of this journal, reviews of notable authors will then focus on special applications and give more detailed insights into single diagnostic fields.

Schlüsselwörter
Durchflusszytometrie · Transfusionsmedizin · Anwendungen

Zusammenfassung
Die Durchflusszytometrie stellt heutzutage in transfusionsmedizinischen Laboratorien eine unverzichtbare Methode zur Bewältigung der täglichen Routine dar. Sie entwickelte sich in den 1960er Jahren aus der Zytophotometrie immobilisierter Zellen und erlaubt im Gegensatz dazu die Analyse vieler tausend Zellen pro Sekunde z. B. aus Blut, Knochenmark oder Zellsuspensionen von festen Organen. Die Einführung fluoreszenzmarkierter, monoklonaler Antikörper erweiterte das Anwendungsspektrum der Durchflusszytometrie erheblich. Mittlerweile lassen sich viele verschiedene zelluläre Eigenschaften simultan auf Einzelzellebene mit hoher Geschwindigkeit und Präzision bestimmen. Durch Einführung interner Standards und Qualitätskontrollen konnte die Messgenauigkeit deutlich erhöht werden. Somit ist die Durchflusszytometrie gleichermaßen für die Patientendiagnostik, die Qualitätskontrolle von Blutprodukten und für wissenschaftliche Fragestellungen in der Transfusionsmedizin geeignet. Viele durchflusszytometrische Applikationen beziehen sich auf die Zählung seltener Ereignisse oder den Nachweis von Zellen mit niedriger Antigenexpression. Deshalb können verschiedene präanalytische und analytische Faktoren (z.B. Erythrozytenlyse, «Gating»-Strategie usw.) die korrekte Bestimmung von Zellzahlen und -funktionen nachhaltig beeinflussen. Die Zeitschrift TRANSFUSION MEDICINE AND HEMOTHERAPY beginnt in dieser Ausgabe mit einer Serie «Durchflusszytometrie in der Transfusionsmedizin». Zur Einleitung dieser Reihe werden die Entwicklung, das Prinzip und die Strategien der Durchflusszytometrie in medizinischen Laboratorien in der vorliegenden Übersichtsarbeit zusammengefasst. Daneben werden derzeitige und künftige Applikationen der Durchflusszytometrie in der Transfusionsmedizin kurz dargestellt, wie z.B. Qualitätskontrolle von Blutprodukten, immunhämatologische Diagnostik, Stammzelltransplantation, sowie adoptive Immuntherapie und weitere therapeutische Applikationen. In nachfolgenden Ausgaben dieser Zeitschrift werden Übersichtsarbeiten dann detaillierter auf einzelne wichtige Applikationen eingehen.

KARGER

Fax +49 761 4 52 07 14
E-mail Information@Karger.de
www.karger.com

Accessible online at:
www.karger.com/tmh

Dr. Burkhard Greve
Institut für Strahlenbiologie
Friedrich-Wilhelms-Universität Münster
Robert-Koch Straße 43, D-48145 Münster
Tel. +49 251 835-25 37, Fax -53 03
E-mail greveb@uni-muenster.de

Development of Flow Cytometry

In the 1950s cytometry started with cytophotometric measurements of light absorption by DNA at 260 nm in cell nuclei and by proteins at 280 nm in the cytoplasm of single cells [1]. The microscope stage with the slide was moved in a sequential trace in front of a small pinhole to provide the integrated optical density as measure for total cellular DNA or protein. This required several minutes per cell. Feulgen DNA staining provided a DNA-specific absorption between 550–570 nm to avoid the use of microscopic UV optics [2].

On the other hand, there were much faster alternatives by which several thousands cells per second could be counted electrically or by light absorption and light scatter when flowing through a fluid-filled measurement chamber [3, 4]. The combination of a flow chamber with photometric absorption measurements [5] and the use of piezo crystals to generate microdroplet streams susceptible to electrostatic deflection [6] provided the basis of the jet in air droplet cell sorter [7] for the preparative enrichment of cell fractions with particular interest. Measurements by absorption were initially considered more promising and sensitive than by fluorescence [8]. The Technicon Hemalog D instrument for absorption measurements of cytochemical stains was developed as a consequence of this attitude [9].

It was, however, convincingly shown that fluorescence measurements were in fact significantly more sensitive than absorption measurements [10, 11]. The Impulse Cytophotometer ICP-11 as the first commercially available fluorescence-based flow cytometer was equipped with a HBO-100 high pressure mercury arc lamp as light source at its appearance in 1969. The promising features of this instrument lead to the conversion of the absorption-based cell spectrometer into the Biophysics/Ortho-Cytofluorograph instrument equipped with an argon 488 nm laser light source for fluorescence excitation and simultaneous light scatter measurements in 1970 [5, 8].

Early European and US authors used a number of different names for the new technology. As an agreement at the 5th American Engineering Foundation Conference on Automated Cytology in 1976, the name flow cytometry was further on used for the new technology. For these nomenclature reasons, a significant amount of the early European work remains usually unconsidered [12].

Work was initially centered around DNA analysis with the first one-parameter DNA and two-parameter DNA/cell protein histogram measurements in ICP-11 instruments [10, 13]. A number of DNA-specific dyes such as ethidium bromide [10], acriflavin/auramin [14], Hoechst 33258 and 33342 dyes [15], propidium iodide [16], mithramycin [17], acridine orange [18], 7-AAD [19] and Dapi [20] were introduced, and numerous cell cycle and tumor cell DNA studies, both clinical and experimental, were performed, which is more recently reviewed by Valet in 2003 [12]. The cell cycle-specific action of cytostatic drugs on malignant cells was demonstrated by specific assays [21] while the use of ethidiumbromide/mithramycin energy transfer [22] provided narrow enough DNA distributions to separate x and y spermatids [23]. Early efforts of computer data analysis concerned monoparametric DNA [24, 25] as well as two-parameter distributions [26].

Factors Influencing Flow Cytometric Analysis

Flow cytometry has become a routine technique for many applications based on standardized laboratory protocols and mainly automated devices. However, there are several technical and methodological difficulties in cell counting which are related to pre-analytic and analytic procedures, possibly affecting accurate analysis. The most important issues are summarized in figure 1.

Influence of Pre-Analytic Sample Preparation

Laboratory workers in this field know that the reliability and accuracy of cell counting depend to a high extent on appropriate pre-analytic sample preparation. Not even the best measuring technique can compensate errors deriving from inaccurate sample pretreatment.

Pipetting

Pipetting is one of the most important factors influencing the counting results, especially if whole blood samples are used. If the common pipetting principle is used to pipette blood, the transferred volume is strongly influenced by adherence of liquid on the outer side of the pipette tip and by aspirating air

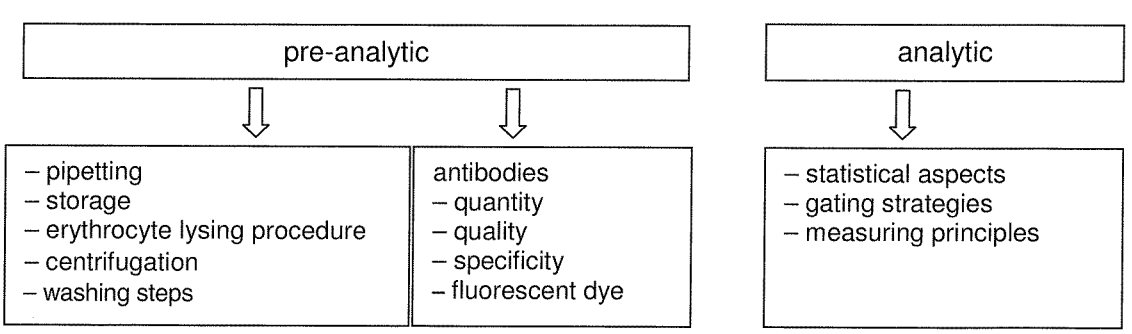

Fig. 1. Factors influencing flow cytometric counting of cells.

bubbles. Adherence of blood can be avoided by cleaning the tip with a tissue paper. But in this case the paper may pull out some blood which reduces the transferred volume. Therefore, the use of reverse pipetting is recommended, where a slight excess of sample is aspirated into the pipette and the precise volume is ejected [27, 28]. Although a small rest remains in the tip of the pipette, the transferred volume is much more exact and not influenced by aspirating air bubbles, which are easily and frequently generated in viscous fluids such as blood. Multiple measurements are recommended to minimize pipetting errors and to improve the quality of analyses.

Storage

Blood is a very special liquid, and its cellular components are sensitive to environmental changes. European guidelines state that leukocyte analyses (like immunophenotyping) should be performed within 18 h [29]. Other standards, like that of the Centers for Disease Control, recommend a maximum storage period of 6 h for the determination of hematologic parameters and 30 h for immunophenotyping [30]. In remote areas samples might need longer time to reach analytic laboratories. Therefore, protocols are modified for these countries by suggesting the use of fixed blood samples which permit longer transportation and storage periods while maintaining sample integrity [31, 32]. However, some cellular populations, e.g. platelets, are very susceptible for environmental impacts and need to be analyzed directly after blood drawing.

Antibodies

The specific binding of antibodies to corresponding antigens is of essential importance in order to assure accurate results. The amount of antibody in a given sample should always distinctly exceed the number of epitopes. Otherwise the target cells may not clearly be discriminated from unspecifically stained and unstained cells. The applied antibodies must have a high specificity to the corresponding epitope; cross-reactivities must be avoided. Antibodies should be suitable for flow cytometric analysis and usually be provided with a CE mark. The quality of antibodies, fluorochromes as well as the conjugation of antibody and fluorochrome must be assured. Exemplary, phycoerythrin is favorable in comparison to fluorescein isothiocyanate or allophycocyanin because of its lower nonspecific binding to dead cells and FC receptors as well as its better discrimination between negative and positive cells and due to its higher quantum yield [33, 34]. The entire sample preparation procedure should not affect the binding efficacy of antibodies to the corresponding epitopes. However, it was demonstrated that the binding of antibodies can be affected by lysis and fixation procedures, resulting in imprecise discrimination between 'positive and negative' cells, thus leading to incorrect cell counts [35].

Erythrocyte Lysing Procedures

The pre-analytic lysis of erythrocytes necessary for analysis of leukocyte subsets may distinctly affect accurate measurements. Although erythrocyte lysing procedures are recommended for all common flow cytometric blood cell counting techniques, it is described as a very critical step in the determination of leukocyte subsets [36, 37]. It is known that erythrocyte lysing procedures affect the determination of progenitor cells in whole blood [36, 38] and cord blood [37] with up to 100% variation in cell counts within identical samples. It was shown that lysis of erythrocytes may also lead to a loss of CD34+ cells and CD45+ cells [39]. Not only the peripheral blood progenitor cells but also CD4+ T cells are affected by the relatively aggressive lysing reagents, resulting in a underrepresentation of T cells, exceeding 10% in some cases [40]. A wide range of side effects such as alteration in morphology and alteration or damage of antigenic epitopes of leukocytes are postulated to account for this phenomenon [38]. However, the impact of the lysing reagent on leukocyte counting and phenotyping may additionally be influenced by the patient's disease and his medical treatment. In fact, the susceptibility of the lymphocyte populations to different lysing procedures varies interindividually and does not represent a constant or calculable parameter in leukocyte subclass enumeration [41]. Therefore, new strategies such as no-lyse sample preparation procedures for the determination of leukocytes and stem cell populations have been evaluated [40, 42].

Influence of Analytical Factors

Beside the pre-analytic factors mentioned above, different apparatus properties, individual technical details, and the disregard of necessary statistical rules may also influence the sensitivity and accuracy in determining specific cells or cell functions.

Single- and Dual-Platform Techniques

The quantification of cells by flow cytometry is mainly based on two different principles. The so-called single-platform method (1-PF) either employs reference beads of known concentrations [43] or measures a defined volume of cell suspensions (absolute volumetric counting) [40]. In dual-platform techniques (2-PF), the absolute values of leukocyte concentrations are calculated using data from a hematology counter in parallel to the flow cytometric determination of the percentage of leukocyte subpopulations [28].

When comparing the determination of low cell concentrations (e.g. the quantification of CD34+ cells), the comparison of laboratory results showed higher mean inter-laboratory coefficients of variation (CVs) for the 2-PF enumeration compared to the 1-PF beads-based technology (28.6 vs. 18.6%) [44]. The requirement of using a hematology counter has been recognized as a significant factor in high inter-laboratory CVs [45]. Although the CVs for 1-PF microbead-based methods are comparatively low, this technique can also lead to artificially high cell counts [46]. This is caused by a decrease in mi-

crobead concentrations as result of vortexing the beads for resuspension in protein-poor media. This effect can be avoided by the addition of protein supplements to the resuspension medium. Beside this, clumping and insufficient resuspension of beads can lead to unpredictable artifacts resulting in falsely high cell values. A collaborative study for determination of CD4+ cells in 102 laboratories showed a mean inter-laboratory CV of 13.7% for the 1-PF microbead-based method and of 23.4% for the 2-PF method [44].

Gating Strategies

Regardless of the measuring technique applied, the gating strategy has been described to have an essential impact on the reproducibility of cell enumeration [27]. Insufficient practical experience as well as the diversity of the currently used gating protocols may lead to a high inter-laboratory CV. Exemplary, significantly different results in the determination of CD34+ cells were obtained when different gating strategies were used. In a multicenter study, 17% of the results from these centers were outside the ±10% range of the median. When all involved centers used the same gating strategy, only 0–7% of results were outside this range [47]. The same difficulties were described for three or four-color flow cytometric immunophenotyping techniques. The gating strategy that combines bright CD45 fluorescence and light scatter properties to determine CD4+ and CD8+ T cells reduces the intra- and inter-laboratory CVs and is therefore recommended [48].

Statistical Limits

For the counting of rare events (like quantification of residual leukocytes in blood components) it is necessary to acquire a sufficient number of positive signals in order to produce statistically valid results. The reduction of measured events per test reduces the sensitivity and insofar the reliability of determinations. The standard error of the number of positive cells per analysis is given by the square root of the number of positive cells. The larger the acquisition the lower the CV [49]. In order to achieve an intra-assay CV of 10%, it is recommended that a minimum of 100 positive events have to be measured [50, 51]. Furthermore, UK NEQAS data showed a high variation in CD45+ cell count between different laboratories when counting significantly less than 50,000 events [52].

Applications for Flow Cytometry in Transfusion Medicine

In the beginning, flow cytometry was primarily used in fundamental medical research, but increasingly is established in quality control of blood components and as advanced diagnostic tool, especially in the field of immunology and immunohematology. In the following, the main applications for flow cytometry in transfusion medicine are given.

i) Quality control of blood products
   - quantification of residual leukocytes in leukoreduced blood components
   - functional investigations of cells or cell fractions in blood components (viability, apoptosis etc.)
   - contaminations of blood components (e. g. with tumor cells or bacteria).

ii) Immunohematologic diagnostics
   - red blood cell (RBC) integrity and immunology
     · detection and quantification of antibodies bound to RBCs
     · detection and quantification of antigens on RBCs
     · detection and quantification of mixed populations of RBCs (chimerism, feto-maternal hemorrhage (FMH))
     · genetic disorders of erythrocytes (paroxysmal nocturnal hemoglobinuria; PNH)
   - platelet integrity and immunology
     · detection and quantification of antibodies against platelets
     · detection and quantification of platelet antigens
     · platelet cross-matching
     · determination of platelet function
   - granulocyte/monocyte integrity and immunology
     · detection of granulocyte function and antigens
     · detection of phagocytosis.

iii) Hematopoietic progenitor cell and solid organ transplantation
   - determination and quantification of hematopoietic progenitor cells in
     . bone marrow
     . peripheral blood
     . cord blood
     · hematopoietic progenitor cell transplants
   - HLA crossmatching
   - quantification of leukocyte subsets.

iv) Adoptive immunotherapy and further therapeutic applications
   - analysis of minimal residual disease (MRD)
     · analysis of viability/apoptosis after manipulation/cryopreservation of cells
     · analysis of dendritic cells
     · analysis of specificity and functionality of T cells
     · analysis of natural killer (NK) cell phenotype and cytotoxicity.

Quality Control of Blood Products

The processing of blood components requires a powerful technique to characterize and enumerate cells and cell fractions in order to meet the high requirements of quality control.
In particular, the quantification of residual leukocytes in blood components is of high medical importance. Current guidelines require the reduction of leukocytes in leukocyte-

depleted blood components to a level below 1×10^6 white blood cells per unit of blood [53]. This seems to be the threshold for transfusion-related complications such as alloimmunization, infections, and mortality [54–57]. Several methods have been described to reliably determine low numbers of leukocytes in blood components, and the role of flow cytometry is growing in this field [58]. Furthermore, functional changes of leukocytes in erythrocyte and platelet concentrates have been investigated by flow cytometry, measuring the apoptosis of cells [59, 60].

With regard to the recent advances in the viral safety of blood products, the attention has shifted to bacterial contaminations as a significant and severe complication in transfusion medicine, especially in platelet transfusion. While several methods are currently exployed for their capacity to screen platelet concentrates for bacterial contaminations, flow cytometry holds the option for such purpose [61]. Also the determination of tumor cells in blood and blood components by flow cytometry has been described [62]. However, the diagnostic value of flow cytometry to detect microbial contaminations or tumor cells in blood components can currently not finally be estimated.

Immunohematologic Diagnostics

RBC Integrity and Immunology

A main application of flow cytometry in transfusion medicine is the diagnostics in immunohematology [63–66]. Although the established agglutination assays are still the most common immunohematologic methods, both the detection and quantification of RBC antigens and antibodies can also be performed by flow cytometric protocols. In particular for special quantitative assays, flow cytometry represents the more convenient and accurate method compared to serologic (agglutination) assays. Numerous studies focus on immunohematologic diagnostics to detect erythrocyte antigens or antibodies, comparing flow cytometry with established agglutination assays [67–73].

The diagnosis of a FMH can be done by the traditional labor-intensive Kleihauer-Betke test [74]. Since flow cytometric analysis allows a more reproducible and sensitive quantification of fetal cells in maternal blood by staining the HbF in fetal RBCs or by labeling of fetal D+RBCs, several groups applied this technique for diagnosis of FMH [75–82].

Moreover, studies demonstrated the feasibility to detect erythrocytic genetic disorders such as the PNH which correlates with the absence or diminishing of glycophosphatidylinositol(GPI)-anchored membrane proteins or other erythrocytic disorders [83–88]. Also, the RBC volume was determined by flow cytometry by exploiting antigen differences between transfused donor RBCs and the recipient's RBCs [89].

Finally, the survival of erythrocytes after transfusion or in patients with autoimmune hemolytic anemia was investigated by labeling RBCs with fluorochromes and subsequent quantification by flow cytometry [90, 91].

Platelet Integrity and Immunology

Flow cytometry was used for phenotyping of platelet-specific antigens [92]. Furthermore, free or cell-bound platelet-specific antibodies were detected and identified by this technique [93–95]. Flow cytometric cross-match assays were introduced in order to predict successful platelet transfusions in immunized patients [96–98]. Also, some special applications such as the determination of platelet engraftment after stem cell transplantation were performed using flow cytometric techniques [99].

In addition, heparin-induced thrombocytopenia (HIT) can be diagnosed by flow cytometric detection of antiheparin/PF4 antibodies. Several publications have evaluated this method for diagnosis of HIT [100–105].

In numerous publications morphologic and functional changes of platelets, e.g. the determination of platelet activation markers in donors, patients or blood components, were investigated by flow cytometry, [106–114]. Finally, platelet activation markers were investigated to determine the biocompatibility of different biomaterials using in vitro models [115–121].

Granulocyte/Monocyte Integrity and Immunology

Flow cytometry was used to identify and characterize granulocyte antigens or antibodies, e.g. in the investigation of transfusion-related acute lung injury (TRALI), in population studies or in the analysis of potential changes during pregnancy [122–127]. Moreover, functional and morphologic changes of circulating neutrophil cells during plateletpheresis were investigated using flow cytometry [128]. Such investigations were also performed to identify the optimal storage conditions for granulocytes [129]. Flow cytometry also allows to detect defined subpopulations of granulocytes such as basophil granulocytes [130]. Finally, the conditions to measure the granulocyte respiratory burst by flow cytometry have been optimized [131, 132].

Some studies used flow cytometry to measure phagocytosis of cells by monocytes or macrophages [133, 134].

Hematopoietic Progenitor Cell and Solid Organ Transplantation

There is an increasing need for accurate determinations of hematopoietic progenitor cells in bone marrow, peripheral blood, and cord blood of patients or donors. Flow cytometric analysis of CD34+ stem cells has turned out to be the method of choice to determine the time point of stem cell apheresis as well as the dosage of the collected progenitor cells. In contrast to the more laborious and time-consuming clonogeneic assays, flow cytometry achieves a much faster and more reproducible analysis of hematopoietic progenitor cells. Several different protocols for the enumeration of CD34+ cells, e.g. the Milan protocol [135], the Milan/Mulhouse protocol [36], the SIHON

protocol [136], the German consensus protocol [137], and the ISHAGE protocol [50] have been published. The latter one is nowadays the most frequently used analysis method and has been described to assure the most reproducible results between centers [47]. Insofar, the counting of CD34 cells has widely been standardized, and the current methods usually meet the requirements of good laboratory practice standards and quality assurance.

Flow cytometry has essentially contributed to the current understanding of stem cell biology. Numerous antigens are co-expressed on progenitor cells and are known to be specific markers for different hematopoietic cell populations, and flow cytometry plays a key role to determine co-expression markers on progenitor cells [138–145].

With the current hype in stem cell biology and transplantation and the extension of the indication for stem cell transplantation to new areas of tissue repair, flow cytometry will presumably again play an important role in the verification of these new treatment options. Recent publications underlined the plasticity of hematopoietic stem cells and suggested differentiation into other tissue cells under optimal conditions. Therefore, flow cytometry will probably become a more powerful tool for monitoring the differentiation of stem and tissue cells by in vitro and in vivo systems [146].

Recently published data suggest that the approach of detecting minimal residual disease by multiparameter flow cytometry is a useful and potentially prognostic relevant tool in patients with hematological diseases [147–150]. Such trials might also be of relevance for quality control of autologous stem cell grafts.

Graft rejection and disease relapse are the major complications occurring after allogeneic stem cell transplantation. Both complications may be associated with dual RBC populations [151]. Several methods for the determination of RBC chimerism after allogeneic hematopoietic stem cell transplantation have been described [152–154].

Immunophenotyping is a common application of flow cytometry and forms the basis for diagnosis and therapy in transplanted and/or immunodeficient patients [155]. Flow cytometric immunophenotyping is nowadays performed using multicolor measurements and therefore allows the simultaneous determination of numerous cell populations and functions [156].

Immunological monitoring of inflammatory processes is possible by flow cytometric determination of monocytic function (HLA-DR+) and phenotype (CD14+ cells) since low HLA-DR expression indicates high risk of infection [157].

Adoptive Immunotherapy and Further Therapeutic Applications

The immune system plays a major role in the progression of malignant diseases [158, 159]. Thus, immunotherapy with cytotoxic cells could represent an interesting alternative therapy that is not cross-reactive with previous treatments administered to patients (chemotherapy, radiation therapy). Several previous reports suggest that some types of cancer are sensitive to immunotherapy and particularly to the lytic activity of activated NK cells or lymphokine-activated killer (LAK) cells. These include melanoma, kidney cancer, acute leukemia, malignant lymphoma, and multiple myeloma [160, 161]. The adoptive transfer of cytotoxic lymphocyte populations in terms of passive immunotherapy may be able to support the patient's immune system. Furthermore, active immunotherapy such as vaccinations with tumor antigen pulsed dendritic cells aiming to elicit a long lasting antitumor response of the patient's immune system. The effect of dendritic cells is largely dependent on the origin (e.g. from stem cells or monocytes) and maturation state of the dendritic cells contained in the transplant.

In addition, recent developments in the sensitive determination of virus-specific T cells using MHC class I-embedded viral peptides and flow cytometry allow to gain significant knowledge about the nature of cellular immune responses against viral infections [162]. These techniques are currently used to monitor the therapeutic effect of adoptive immunotherapies in viral infections after stem cell transplantation in hematologic malignancies [163] and will ultimately lead to the development of new cellular therapies in this life-threatening disease [164, 165].

Moreover, new test systems, e.g. intracellular cytokine staining and tetramer staining, enable the analysis of the functionality and the specificity of defined cell populations such as CD4+ or CD8+ T cells by flow cytometry. These methods are also essential in the monitoring of adoptive immunotherapy [165–167].

The cytotoxic function of immune effector cells can be readily determined using flow cytometry-based test systems, and nowadays avoids the use of radioactive substances [168, 169]. Additionally, further flow cytometric techniques such as the evaluation of viability or apoptosis in hematopoietic progenitor transplants after cryopreservation with dyes like 7-amino actinomycin (7-AAD) or Syto16 [170, 171] or flow cytometric assays to determine T-cell-mediated cytotoxic activity [172, 173] in the context of immunotherapeutic clinical trials became routinely applied diagnostic measures. Therefore, flow cytometry has definitely made a major impact on the development of both active and passive immunotherapeutic approaches and their development from bench to bedside.

However, the application of cells for immunotherapy needs a thorough quality control in terms of phenotype, purity, and activity of the cell transplant in order to maximize the potential therapeutic effect and at the same time to minimize side effects such as the development of graft-versus-host disease. This quality control is therefore a prerequisite for the clinical application. Flow cytometry certainly represents the state of the art method to determine the phenotype and function of dendritic cells and other immune cells [174–177].

In summary, flow cytometry represents a highly innovative technique for many common diagnostic and scientific fields in transfusion medicine. Finally, it is the tool of choice to develop and optimize new cellular and immunotherapeutic trials.

References

1 Caspersson TO: Cell Growth and Cell Function: A Cytochemical Study. New York, Norton & Co, 1950.
2 Chieco P, Derenzini M: The Feulgen reaction 75 years on. Histochem Cell Biol 1999;111:345–358.
3 Coulter WH: Means for counting particles suspended in a fluid. US patent 2,656,508, 1953.
4 Crosland-Taylor PJ: A device for counting small particles suspended in a fluid through a tube. Nature 1953;171:37–38.
5 Kamentsky LA, Melamed MR, Derman H: Spectrophotometer: New instrument for ultrarapid cell analysis. Science 1965;150:630–631.
6 Sweet RG: High frequency recording with electrostatically deflected in jets. Rev Sci Instrum 1965;36: 131–136.
7 Fulwyler MJ: Electronic separation of biological cells by volume. Science 1965;150:910–911.
8 Kamentsky LA: Discussion remarks; in Evans DMD (ed): Cytology Automation, Proceedings 2nd Tenovus Symposium, Cardiff October 24–25, 1968. Vol. 55. Edinburgh, Livingstone, 1970, p 196.
9 Simmons A, Leaverton P, Elbert G: Normal laboratory values for differential white cell counts established by manual and automated cytochemical methods (Hemalog D-TM). J Clin Pathol 1974;27: 55–58.
10 Dittrich W, Göhde W: Impulsfluorimetrie bei Einzelzellen in Suspensionen. Z Naturforsch 1969; 24b:221–228.
11 Göhde W: Automatisches Meß- und Zählgerät für die Teilchen einer Dispersion. Patent DE 1815352, 1968.
12 Valet G:Past and present concepts in flow cytometry: A European perspective. J Biol Regul Homeost Agents 2003;17:213–222.
13 Göhde W, Dittrich W: Simultane Impulsfluorimetrie des DNS- und Proteingehaltes von Tumorzellen. Z Anal Chem 1970;252:328–330.
14 Van Dilla MA, Trujillo TT, Mullaney PF, Coulter JR: Cell microfluorometry: A method for rapid fluorescence measurement. Science 1969;163:1213–1214.
15 Lämmler G, Schütze HR: Vital-Fluorchromierung tierischer Zellkerne mit einem neuen Fluorochrom. Naturwissenschaften 1969;56:286.
16 Crissman HA, Steinkamp JA: Rapid, simultaneous measurement of DNA, protein, and cell volume in single cells from large mammalian cell populations. J Cell Biol 1973;59:766–771.
17 Crissman HA, Tobey RA: Cell-cycle analysis in 20 minutes. Science 1974;184:1297–1298.
18 Darzynkiewicz Z, Traganos F, Melamed MR: New cell cycle compartments identified by multiparameter flow cytometry. Cytometry 1980;1:98–108.
19 Zelenin AV, Poletaev AI, Stepanova NG, et al: 7-amino-actinomycin D as a specific fluorophore for DNA content analysis by laser flow cytometry. Cytometry 1984;5:348–354.
20 Göhde W, Schumann J, Zante J: The use of DAPI in pulse cytophotometry; in Lutz D (ed): Third International Symposium on Pulse-Cytophotometry. Gent, European Press, 1978, pp 229–232.
21 Göhde W, Dittrich W: Cytostatische Wirkung von Daunomycin im Impulscytometrie Test. Arzneimittelforschung 1971;21:1656–1658.
22 Zante J, Schumann J, Barlogie B, Göhde W, Buchner T: New preparation and staining procedures for specific and rapid analysis of DNA distributions; in Göhde W Schumann J, Buchner T (edsSecond International Symposium on Pulse Cytophotometry. Application in Cancer Research and Hematology with Special Reference to Cell Kinetics. Gent, European Press, 1976, pp 97–106.
23 Meistrich ML, Göhde W, White RA, Schumann J: Resolution of X and Y spermatids by pulse cytophotometry. Nature 1978;274:821–823.
24 Dean PN, Anderson EC: The rate of DNA synthesis during S-phase by mammalian cells in vitro; in Haanen CAM, Hillen HFP, Wessels JMC (eds): First International Symposium on Pulse-Cytophotometry. Gent: European Press Medikon, 1975, pp 77–86.
25 Baisch H, Göhde W, Linden WA: Mathematical analysis of ICP-data to determine the fraction of cells in the various phases of cell cycle; in Haanen CAM, Hillen HFP, Wessels JMC (eds): First International Symposium on Pulse-Cytophotometry. Gent, European Press Medikon, 1975, pp 68–76.
26 Baisch H, Göhde W: Mathematical analysis of DNA-protein two parameter pulse-cytophotometric data; in Göhde W, Schumann J, Buchner T (eds): Second International Symposium on Pulse Cytophotometry. Application in Cancer Research and Hematology with Special Reference to Cell Kinetics. Gent, European Press, 1976, pp 71–78.
27 Barnett D, Janossy G, Lubenko A, Matutes E, Newland A, Reilly JT: Guideline for the flow cytometric enumeration of CD34+ haematopoietic stem cells. Prepared by the CD34+ haematopoietic stem cell working party. General Haematology Task Force of the British Committee for Standards in Haematology. Clin Lab Haematol 1999;21:301–308.
28 Brando B, Barnett D, Janossy G, Mandy F, Autran B, Rothe G, Scarpati B, D'Avanzo G, D'Hautcourt JL, Lenkei R, Schmitz G, Kunkl A, Chianese R, Papa S, Gratama JW: Cytofluorometric methods for assessing absolute numbers of cell subsets in blood. European Working Group on Clinical Cell Analysis. Cytometry 2000;42:327–346.
29 CD4+ T Lymphocyte Working Party. Members of the General Haematology Task Force of BCSH: Guidelines for the enumeration of CD4+ T lymphocytes in immunosuppressed individuals. Clin Laby Haematol 1997;19:231–241.
30 Centers for Disease Control and Prevention.: Guidelines for the Performance of CD4+ T-Cell Determinations in Persons with Human Immunodeficiency Virus Infection. MMWR Recomm Rep 1992;41:1–17.
31 Jani V, Janossy G, Iqbal A, Mhalu FS, Lyamuya EF, Biberfeld G, Glencross DK, Scott L, Reilly JT, Granger V, Barnett D: Affordable CD4+ T cell counts by flow cytometry. II. The use of fixed whole blood in resource-poor settings. J Immunol Methods 2001;257:145–154.
32 Harrison GM, Bennett AJ, Moody M, Read GF, Williams PE: Use of formalin-fixed, propidium iodide-stained human leukocytes as a standard for enumerating CD4+ T lymphocytes in a single-platform assay. Clin Diagn Lab Immunol 2001;8:397–401.
33 Gratama JW, van der Linden R, van der Holt B, Bolhuis RL van de Winkel JG: Analysis of factors contributing to the formation of mononuclear cell aggregates ('escapees') in flow cytometric immunophenotyping. Cytometry 1997;29:250–260.
34 Johnsen HE: Report from a Nordic workshop on CD34+ cell analysis: Technical recommendations for progenitor cell enumeration in leukapheresis from multiple myeloma patients. Nordic Myeloma Study Group Laboratories. J Hematother 1995;4: 21–28.
35 Macey MG, McCarthy DA, van Agthoven A, Newland AC: How should CD34+ cells be analysed? A study of three classes of antibody and five leucocyte preparation procedures. J Immunol Methods 1997;204:175–188.
36 Bender JG, Unverzagt K, Walker D: Guidelines for determination of CD34+ cells by flow cytometry: Application to the harvesting and transplantation of peripheral blood stem cells; in Wunder E, Sovalat H, Henon P, Serke S (eds): Hematopoietic Stem Cells. Dayton, Alpha Med Press, 1994, pp 31–34.
37 Cassens U, Gutensohn K, Garritsen H, Kelsch R, Kuehnl P, Sibrowski W: The influence of different erythrocyte lysing procedures on flow cytometric determination of CD34+ cells in umbilical cord blood transplants. Transfus Med 1998;8:111–118.
38 Kreissig C, Kirsch A, Serke S: Characterization and measurement of CD34-expressing hematopoietic cells. J Hematother 1994;3:263–289.
39 Greve B, Beller C, Cassens U, Sibrowski W, Severin E, Göhde W: High-grade loss of leukocytes and hematopoietic progenitor cells caused by erythrocyte-lysing procedures for flow cytometric analyses. J Hematother Stem Cell Res 2003;12:321–330.
40 Greve B, Cassens U, Westerberg C, Göhde W Jr, Sibrowski W, Reichelt D, Göhde W: A new no-lyse, no-wash flow-cytometric method for the determination of CD4 T cells in blood samples. Transfus Med Hematother 2003;30:8–13.
41 Göhde W, Cassens U, Lehman LE, Traoré Y, Göhde W Jr, Perkes P, Westerberg C, Greve B: Individual patient-dependent influence of erythrocyte lysing procedures on flow-cytometric analysis of leukocyte subpopulations. Transfus Med Hematother 2003;30:165–170.
42 Alvarez-Larran A, Jover L, Marin P, Petriz J: A multicolor, no-lyse no-wash assay for the absolute counting of CD34+ cells by flow cytometry. Cytometry 2002;50:249–253.
43 Reilly JT, Barnett D: UK NEQAS for leucocyte immunophenotyping: The first 10 years. J Clin Pathol 2001;54:508–511.
44 Barnett D, Granger V, Whitby L, Storie I, Reilly JT: Absolute CD4+ T-lymphocyte and CD34+ stem cell counts by single-platform flow cytometry: The way forward. Br J Haematol 1999;106:1059–1062.
45 O'Gorman MR, Nicholson JK: Adoption of single-platform technologies for enumeration of absolute T-lymphocyte subsets in peripheral blood. Clin Diagn Lab Immunol 2000;7:333–335.
46 Brando B, Göhde W Jr, Scarpati B, D'Avanzo G: The 'vanishing counting bead' phenomenon: effect on absolute CD34+ cell counting in phosphate-buffered saline-diluted leukapheresis samples. Cytometry 2001;43:154–160.
47 Chang A, Ma DD: The influence of flow cytometric gating strategy on the standardization of CD34+ cell quantitation: an Australian multicenter study. Australian BMT Scientists Study Group. J Hematother 1996;5:605–616.
48 Schnizlein-Bick CT, Mandy FF, O'Gorman MR, Paxton H, Nicholson JK, Hultin LE, Gelman RS, Wilkening CL, Livnat D: Use of CD45 gating in three and four-color flow cytometric immunophenotyping: Guideline from the National Institute of Allergy and Infectious Diseases, Division of AIDS. Cytometry 2002;50:46–52.
49 Wunder E, Sovalat H, Fritsch G, Silvestri F, Henon P, Serke S: Report on the European Workshop on Peripheral Blood Stem Cell Determination and Standardization – Mulhouse, France, February 6–8 and 14–15, 1992. J Hematother 1992;1:131–142.

50 Sutherland DR, Anderson L, Keeney M, Nayar R, Chin-Yee I: The ISHAGE guidelines for CD34+ cell determination by flow cytometry. International Society of Hematotherapy and Graft Engineering. J Hematother 1996;5:213–226.

51 Storie I, Sawle A, Goodfellow K, Whitby L, Granger V, Reilly JT, Barnett D: Flow rate calibration I: A novel approach for performing absolute cell counts. Cytometry 2003;55B:1–7.

52 Barnett D, Granger V, Storie I, Peel J, Pollitt R, Smart T, Reilly JT: Quality assessment of CD34+ stem cell enumeration: Experience of the United Kingdom National External Quality Assessment Scheme (UK NEQAS) using a unique stable whole blood preparation. Br J Haematol 1998;102:553–565.

53 Council of Europe: Guide to the Preparation, Use and Quality Assurance of Blood Components. Strasbourg, Council of Europe, 2001:103–126.

54 Jensen LS, Kissmeyer-Nielsen P, Wolff B, Qvist N: Randomised comparison of leucocyte-depleted versus buffy-coat-poor blood transfusion and complications after colorectal surgery. Lancet 1996;348: 841–845.

55 The Trial to Reduce Alloimmunization to Platelets Study Group: Leukocyte reduction and ultraviolet B irradiation of platelets to prevent alloimmunization and refractoriness to platelet transfusions. N Engl J Med 1997;337:1861–1869.

56 van de Watering LM, Hermans J, Houbiers JG, van den Broek PJ, Bouter H, Boer F, Harvey MS, Huysmans HA, Brand A: Beneficial effects of leukocyte depletion of transfused blood on postoperative complications in patients undergoing cardiac surgery: A randomized clinical trial. Circulation 1998; 97:562–568.

57 Vamvakas EC: Meta-analysis of randomized controlled trials of the efficacy of white cell reduction in preventing HLA-alloimmunization and refractoriness to random-donor platelet transfusions. Transfus Med Rev 1998;12:258–270.

58 Dzik S: Principles of counting low numbers of leukocytes in leukoreduced blood components. Transfus Med Rev 1997;11:44–55.

59 Frabetti F, Musiani D, Marini M, Fanelli C, Coppola S, Ghibelli L, Tazzari PL, Bontadini A, Tassi C, Conte R: White cell apoptosis in packed red cells. Transfusion 1998;38:1082–1089.

60 Frabetti F, Tazzari PL, Musiani D, Bontadini A, Matteini C, Roseti L, Tassi C, Viggiani M, Marini M, Conte R: White cell apoptosis in platelet concentrates. Transfusion 2000;40:160–168.

61 Seghatchian J, Krailadsiri P, Beard M: Does bacterial contamination of platelet concentrates influence the leucocyte content and the rate of platelet storage lesion? Transfus Sci 2000;22:139–143.

62 Terstappen LW, Rao C, Gross S, Kotelnikov V, Racilla E, Uhr J, Weiss A : Flow cytometry – principles and feasibility in transfusion medicine. Enumeration of epithelial derived tumor cells in peripheral blood. Vox Sang 1998;74(suppl 2):269–274.

63 Boval B: Flow cytometry: Applications in transfusion medicine. Transfus Clin Biol 2000;7(suppl 1): 63s–68s.

64 Garratty G, Arndt PA: Applications of flow cytofluorometry to red blood cell immunology. Cytometry 1999;38:259–267.

65 Freedman J, Lazarus AH: Applications of flow cytometry in transfusion medicine. Transfus Med Rev 1995;9:87–109.

66 Davis BH: Diagnostic utility of red cell flow cytometric analysis. Clin Lab Med 2001;21:829–840.

67 Wagner F: Identification of recipient Rh phenotype in a chronically transfused child by two-colour immunofluorescence. Transfus Med 1994;4:205–208.

68 Austin EB, McIntosh Y, Hodson C, Lee D: Anti-D quantification by flow cytometry: An alternative to the AutoAnalyser? Transfus Med 1995;5:203–208.

69 Stroncek DF, Njoroge JM, Procter JL, Childs RW, Miller J: A preliminary comparison of flow cytometry and tube agglutination assays in detecting red blood cell-associated C3d. Transfus Med 2003;13: 35–41.

70 Nicholson G, Lawrence A, Ala FA, Bird GW: Semi-quantitative assay of D antigen site density by flow cytometric analysis. Transfus Med 1991;1:87–90.

71 Lynen R, Krone O, Legler TJ, Kohler M, Mayr WR: A newly developed gel centrifugation test for quantification of RBC-bound IgG antibodies and their subclasses IgG1 and IgG3: comparison with flow cytometry. Transfusion 2002;42:612–618.

72 Lynen R, Neuhaus R, Schwarz DW, Simson G, Riggert J, Mayr WR, Kohler M: Flow cytometric analyses of the subclasses of red cell IgG antibodies. Vox Sang 1995;69:126–130.

73 Wagner T, Resch B, Legler TJ, Mossier C, Helmberg W, Kohler M, Lanzer G: Severe HDN due to anti-Ce that required exchange tranfusion. Transfusion 2000;40:571–574.

74 Kleihauer E, Braun H, Betke K: Demonstration von fetalem Haemoglobin in den Erythrocyten eines Blutausstriches. Klin Wochenschr 1957;35: 637–638.

75 Chen JC, Davis BH, Wood B, Warzynski MJ: Multicenter clinical experience with flow cytometric method for fetomaternal hemorrhage detection. Cytometry 2002;50:285–290.

76 Kennedy GA, Shaw R, Just S, Bryson G, Battistutta F, Rowell J, Williams B: Quantification of fetomaternal haemorrhage (FMH) by flow cytometry: Anti-fetal haemoglobin labelling potentially underestimates massive FMH in comparison to labelling with anti-D. Transfus Med 2003;13:25–33.

77 Greiss MA, Armstrong-Isher SS, Perera WS, Brown PM, Urbaniak SJ: Semiautomated data analysis of flow cytometric estimation of fetomaternal hemorrhage in D- women. Transfusion 2002;42: 1067–1078.

78 Kumpel BM: Labeling D+ RBCs for flow cytometric quantification of fetomaternal hemorrhage after the RBCs have been coated with anti-D. Transfusion 2001;41:1059–1063.

79 Kumpel BM: Analysis of factors affecting quantification of fetomaternal hemorrhage by flow cytometry. Transfusion 2000;40:1376–1383.

80 Lubenko A, Williams M, Johnson A, Pluck J, Armstrong D, MacLennan S: Monitoring the clearance of fetal RhD-positive red cells in FMH following RhD immunoglobulin administration. Transfus Med 1999;9:331–335.

81 Johnson PR, Tait RC, Austin EB, Shwe KH, Lee D: Flow cytometry in diagnosis and management of large fetomaternal haemorrhage. J Clin Pathol 1995;48:1005–1008.

82 Howarth DJ, Robinson FM, Williams M, Norfolk DR: A modified Kleihauer technique for the quantification of foetomaternal haemorrhage. Transfus Med 2002;12:373–378.

83 Doukas MA, Fleming D, Jennings D: Identical twin marrow transplantation for venous thrombosis in paroxysmal nocturnal hemoglobinuria; long-term complete remission as assessed by flow cytometry. Bone Marrow Transplant 1998;22:717–721.

84 Marsh JC, Elebute MO: Stem cells in paroxysmal nocturnal haemoglobinuria and aplastic anaemia: Increasing evidence for overlap of haemopoietic defect. Transfus Med 2003;13:377–386.

85 Matthes T, Tullen E, Poole J, Banks J, Nagy M, Stelling MJ, Boehlen F, Michel M, Beris P, Hustinx H, Crew V, Daniels G: Acquired and transient RBC CD55 deficiency (Inab phenotype) and anti-IFC. Transfusion 2002;42:1448–1457.

86 Richards SJ, Rawstron AC, Hillmen P: Application of flow cytometry to the diagnosis of paroxysmal nocturnal hemoglobinuria. Cytometry 2000; 42:223–233.

87 Navenot JM, Muller JY, Blanchard D: Investigation of the survival of paroxysmal nocturnal hemoglobinuria red cells through the immunophenotyping of reticulocytes. Transfusion 1998;38:337–342.

88 Krauss JS: Laboratory diagnosis of paroxysmal nocturnal hemoglobinuria. Ann Clin Lab Sci 2003; 33:401–406.

89 Fisher J, Matthes JW, Wynn R, Al-Ismail SA, Hoy TG, Wardrop CA, Williams JL: Determination of red cell volume in infants needing blood transfusion. Transfus Med 2000;10:219–224.

90 Zeiler T, Muller JT, Hasse C, Kullmer J, Kretschmer V: Flow cytometric determination of RBC survival in autoimmune hemolytic anemia. Transfusion 2001;41:493–498.

91 Kumpel BM, Austin EB, Lee D, Jackson DJ, Judson PA, Chapman GE: Comparison of flow cytometric assays with isotopic assays of (51)chromium-labeled cells for estimation of red cell clearance or survival in vivo. Transfusion 2000;40:228–239.

92 Watkins NA, Armour KL, Smethurst PA, Metcalfe P, Scott ML, Hughes DL, Smith GA, Williamson LM, Clark MR, Ouwehand WH: Rapid phenotyping of HPA-1a using either diabody-based hemagglutination or recombinant IgG1-based assays. Transfusion 1999;39:781–789.

93 McFarland JG: Detection and identification of platelet antibodies in clinical disorders. Transfus Apheresis Sci 2003;28:297–305.

94 Levin MD, de Vries W, de Veld J, Doekharan D, van der Holt B, van 't Veer MB: Platelet-bound immunogloblins before and after platelet transfusion: Measurement of in vivo binding. Br J Haematol 1999;104:397–402.

95 Sintnicolaas K, de Vries W, van der Linden R, Gratama JW, Bolhuis RL: Simultaneous flow cytometric detection of antibodies against platelets, granulocytes and lymphocytes. J Immunol Methods 1991;142:215–222.

96 Sintnicolaas K, Lowenberg B: A flow cytometric platelet immunofluorescence crossmatch for predicting successful HLA matched platelet transfusions. Br J Haematol 1996;92:1005–1010.

97 Kohler M, Dittmann J, Legler TJ, Lynen R, Humpe A, Riggert J, Neumeyer H, Pies A, Panzer S, Mayr WR: Flow cytometric detection of platelet-reactive antibodies and application in platelet crossmatching. Transfusion 1996;36:250–255.

98 Skogen B, Christiansen D, Husebekk A: Flow cytometric analysis in platelet crossmatching using a platelet suspension immunofluorescence test. Transfusion 1995;35:832–836.

99 Figuerres E, Olszewski M, Kletzel M: A flow cytometric technique using thiazole orange to detect platelet engraftment following pediatric stem-cell transplants. Cytotherapy 2001;3:277–283.

100 Sobas F, Colin C, Ffrench P, Trzeciak MC, Dechavanne M, Negrier C: In vitro tests for assessing heparin-induced thrombocytopenia in patients after elective hip replacement. A medico-economical evaluation. Int J Technol Assess Health Care 2002; 18:555–565.

101 Poley S, Mempel W: Laboratory diagnosis of heparin-induced thrombocytopenia: Advantages of a functional flow cytometric test in comparison to the heparin-induced platelet-activation test. Eur J Haematol 2001;66:253–262.
102 Hughes M, Hayward CP, Warkentin TE, Horsewood P, Chorneyko KA, Kelton JG: Morphological analysis of microparticle generation in heparin-induced thrombocytopenia. Blood 2000;96:188–194.
103 Tomer A, Masalunga C, Abshire TC: Determination of heparin-induced thrombocytopenia: A rapid flow cytometric assay for direct demonstration of antibody-mediated platelet activation. Am J Hematol 1999;61:53–61.
104 Tazzari PL, Ricci F, Vitale M, Malferrari F, Salama A, Schwind P, Conte R: Heparin-induced thrombocytopenia: Detection of antiheparin/PF4 antibodies by means of heparin/PF4-coated beads and flow cytometry. Transfus Med 2002;12:193–198.
105 Vitale M, Tazzari P, Ricci F, Mazza MA, Zauli G, Martini G, Caimi L, Manzoli FA, Conte R: Comparison between different laboratory tests for the detection and prevention of heparin-induced thrombocytopenia. Cytometry 2001;46:290–295.
106 Rand ML, Leung R, Packham MA: Platelet function assays. Transfus Apheresis Sci 2003;28:307–317.
107 Peerschke EI: The laboratory evaluation of platelet dysfunction. Clin Lab Med 2002;22:405–420.
108 Hickerson DH, Bode AP: Flow cytometry of platelets for clinical analysis. Hematol Oncol Clin North Am 2002;16:421–454.
109 Harrison P: Progress in the assessment of platelet function. Br J Haematol 2000;111:733–744.
110 Michelson AD, Furman MI: Laboratory markers of platelet activation and their clinical significance. Curr Opin Hematol 1999;6:342–348.
111 Rinder HM: Platelet function testing by flow cytometry. Clin Lab Sci 1998;11:365–372.
112 Michelson AD: Flow cytometry: A clinical test of platelet function. Blood 1996;87:4925–4936.
113 Kao S, Turner NA, Moake JL, McIntire LV: A novel flow cytometric analysis for platelet activation on immobilized von Willebrand factor or fibrillar collagen. J Thromb Haemost 2003;1:347–354.
114 Rinder HM, Snyder EL, Bonan JL, Napychank PA, Malkus H, Smith BR: Activation in stored platelet concentrates: Correlation between membrane expression of P-selectin, glycoprotein IIb/IIIa, and beta-thromboglobulin release. Transfusion 1993;33:25–29.
115 Keldenich S, Kopp R, Kirschfink M, Klein B, Henseler A, Thelen H, Oedekoven B, Mottaghy K: Application of a new dynamic flow model for investigating the biocompatibility of modified surfaces. Asaio J 2000;46:134–141.
116 Tarnok A, Mahnke A, Muller M, Zotz RJ: Rapid in vitro biocompatibility assay of endovascular stents by flow cytometry using platelet activation and platelet-leukocyte aggregation. Cytometry 1999;38:30–39.
117 Baker LC, Davis WC, Autieri J, Watach MJ, Yamazaki K, Litwak P, Wagner WR: Flow cytometric assays to detect platelet activation and aggregation in device-implanted calves. J Biomed Mater Res 1998;41:312–321.
118 Gutensohn K, Beythien C, Bau J, Meinertz T, Kuehnl P: Flow cytometric analysis of coronary stent-induced alterations of platelet antigens in an in vitro model. Thromb Res 1997;86:49–56.
119 Snyder TA, Watach MJ, Litwak KN, Wagner WR: Platelet activation, aggregation, and life span in calves implanted with axial flow ventricular assist devices. Ann Thorac Surg 2002;73:1933–1938.
120 Gutensohn K, Beythien C, Bau J, Fenner T, Grewe P, Koester R, Padmanaban K, Kuehnl P: In vitro analyses of diamond-like carbon coated stents. Reduction of metal ion release, platelet activation, and thrombogenicity. Thromb Res 2000;99:577–585.
121 Gutensohn K, Kuehnl P: The role of flow cytometry in improving biocompatibility in transfusion medicine. Transfus Sci 1998;19:17–25.
122 Kao GS, Wood IG, Dorfman DM, Milford EL, Benjamin RJ: Investigations into the role of anti-HLA class II antibodies in TRALI. Transfusion 2003;43:185–191.
123 Stroncek DF, Shankar R, Litz C, Clement L: The expression of the NB1 antigen on myeloid precursors and neutrophils from children and umbilical cords. Transfus Med 1998;8:119–123.
124 Davoren A, Curtis BR, Shulman IA, Mohrbacher AF, Bux J, Kwiatkowska BJ, McFarland JG, Aster RH: TRALI due to granulocyte-agglutinating human neutrophil antigen-3a (5b) alloantibodies in donor plasma: A report of 2 fatalities. Transfusion 2003;43:641–645.
125 Caruccio L, Bettinotti M, Matsuo K, Sharon V, Stroncek D: Expression of human neutrophil antigen-2a (NB1) is increased in pregnancy. Transfusion 2003;43:357–363.
126 Flesch BK, Doose S, Siebert R, Ntambi E, Neppert J: FCGR3 variants and expression of human neutrophil antigen-1a, -1b, and -1c in the populations of northern Germany and Uganda. Transfusion 2002;42:469–475.
127 Taniguchi K, Kobayashi M, Harada H, Hiraoka A, Tanihiro M, Takata N, Kimura A: Human neutrophil antigen-2a expression on neutrophils from healthy adults in western Japan. Transfusion 2002; 42:651–657.
128 Western KH, Videm V: Donor neutrophil function after plateletpheresis. Transfusion 2000;40:1414–1418.
129 Chun H, Cipolone K, Procter J, Stroncek DF: Granulocyte storage and antigen stability. Transfusion 1999;39:983–990.
130 Gane P, Pecquet C, Lambin P, Abuaf N, Leynadier F, Rouger P: Flow cytometric evaluation of human basophils. Cytometry 1993;14:344–348.
131 Wolber RA, Duque RE, Robinson JP, Oberman HA: Oxidative product formation in irradiated neutrophils. A flow cytometric analysis. Transfusion 1987;27:167–170.
132 Vowells SJ, Sekhsaria S, Malech HL, Shalit M, Fleisher TA: Flow cytometric analysis of the granulocyte respiratory burst: A comparison study of fluorescent probes. J Immunol Methods 1995;178: 89–97.
133 Lim J, Kim Y, Han K, Kim M, Lee KY, Kim WI, Shim SI, Kim BK, Kang CS: Flow cytometric monocyte phagocytic assay for predicting platelet transfusion outcome. Transfusion 2002;42:309–316.
134 Wiener E, Abeyakoon O, Benchetrit G, Lyall M, Keler T and Rodeck CH: Anti-HPA-1a-mediated platelet phagocytosis by monocytes in vitro and its inhibition by Fc gamma receptor (FcgammaR) reactive reagents. Eur J Haematol 2003;70:67–74.
135 Siena S, Bregni M, Brando B, Belli N, Ravagnani F, Gandola L, Stern AC, Lansdorp PM, Bonadonna G, Gianni AM: Flow cytometry for clinical estimation of circulating hematopoietic progenitors for autologous transplantation in cancer patients. Blood 1991;77:400–409.
136 Gratama JW, Kraan J, Levering W, Van Bockstaele DR, Rijkers GT, Van der Schoot CE: Analysis of variation in results of CD34+ hematopoietic progenitor cell enumeration in a multicenter study. Cytometry 1997;30:109–117.
137 Gutensohn K, Serke S, Kretschmer V: Durchflußzytometrische Analyse CD34 exprimierender hämatopoetischer Zellen in Blut und Zytaferese-produkten. Infusionsther Transfusionsmed 1996;23 (suppl. 2):1–23.
138 Kinniburgh D, Russell NH: Comparative study of CD34-positive cells and subpopulations in human umbilical cord blood and bone marrow. Bone Marrow Transplant 1993;12:489–494.
139 Inaba T, Shimazaki C, Tatsumi T, Yamagata N, Hirata T, Goto H, Fujita N, Nakagawa M: Expression of differentiation-associated antigens and adhesion molecules on CD34-positive cells harvested from peripheral blood and bone marrow. Prog Clin Biol Res 1994;389:331–337.
140 De Bruyn C, Delforge A, Bron D, Bernier M, Massy M, Ley P, de Hemptinne D, Stryckmans P: Comparison of the coexpression of CD38, CD33 and HLA-DR antigens on CD34+ purified cells from human cord blood and bone marrow. Stem Cells 1995;13:281–288.
141 Inaba T, Shimazaki C, Hirata T, Ashihara E, Okawa K, Oku N, Goto H, Fujita N, Tsuji H, Nakagawa M: Phenotypic differences of CD34-positive stem cells harvested from peripheral blood and bone marrow obtained before and after peripheral blood stem cell collection. Bone Marrow Transplant 1994;13:527–532.
142 D'Arena G, Cascavilla N, Musto P, Greco M, Di Mauro L, Carella AM, Dello Iacono N, Carotenuto M: Flow cytometric characterization of CD34+ hematopoietic progenitor cells in mobilized peripheral blood and bone marrow of cancer patients. Haematologica 1996;81:216–223.
143 Dercksen MW, Rodenhuis S, Dirkson MK, Schaasberg WP, Baars JW, van der Wall E, Slaper-Cortenbach IC, Pinedo HM, Von dem Borne AE, van der Schoot CE, et al: Subsets of CD34+ cells and rapid hematopoietic recovery after peripheral-blood stem-cell transplantation. J Clin Oncol 1995;13:1922–1932.
144 Millar BC, Millar JL, Shepherd V, Blackwell P, Porter H, Cunningham D, Judson I, Treleaven J, Powles RL, Catovsky D: The importance of CD34+/CD33– cells in platelet engraftment after intensive therapy for cancer patients given peripheral blood stem cell rescue. Bone Marrow Transplant 1998;22:469–475.
145 Humpe A, Riggert J, Koch S, Legler TJ, Munzel U, Kohler M: Prospective, randomized, sequential, crossover trial of large-volume vs. normal-volume leukapheresis procedures: effects on subpopulations of CD34(+) cells. J Clin Apheresis 2001;16: 109–113.
146 Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, Schumichen C, Nienaber CA, Freund M, Steinhoff G: Autologous bone-marrow stem-cell transplantation for myocardial regeneration. Lancet 2003;361:45–46.
147 Cabezud E, Matutes E, Ramrattan M, Morilla R, Catovsky D: Analysis of residual disease in chronic lymphocytic leukemia by flow cytometry. Leukemia 1997;11:1909–1914.
148 Rawstron AC, Kennedy B, Evans PA, Davies FE, Richards SJ, Haynes AP, Russell NH, Hale G, Morgan GJ, Jack AS, Hillmen P: Quantitation of minimal disease levels in chronic lymphocytic leukemia using a sensitive flow cytometric assay improves the prediction of outcome and can be used to optimize therapy. Blood 2001;98:29–35.

149 Garcia Vela JA, Delgado I, Garcia Alonso L, Monteserin MC, Benito L, Ona F, Lastra AM: Detection of minimal residual disease in B-cell chronic lymphocytic leukaemia by flow cytometry. Br J Haematol 1997;99:464–465.
150 Kern W, Danhauser-Riedl S, Ratei R, Schnittger S, Schoch C, Kolb HJ, Ludwig WD, Hiddemann W, Haferlach T: Detection of minimal residual disease in unselected patients with acute myeloid leukemia using multiparameter flow cytometry for definition of leukemia-associated immunophenotypes and determination of their frequencies in normal bone marrow. Haematologica 2003;88: 646–653.
151 David B, Bernard D, Navenot JM, Muller JY, Blanchard D: Flow cytometric monitoring of red blood cell chimerism after bone marrow transplantation. Transfus Med 1999;9:209–217.
152 Arkesteijn GJ, Erpelinck SL, Martens AC, Hagenbeek A: Chromosome specific DNA hybridization in suspension for flow cytometric detection of chimerism in bone marrow transplantation and leukemia. Cytometry 1995;19:353–360.
153 Jurlander J, Caligiuri MA, Ruutu T, Baer MR, Strout MP, Oberkircher AR, Hoffmann L, Ball ED, Frei-Lahr DA, Christiansen NP, Block AM, Knuutila S, Herzig GP, Bloomfield CD: Persistence of the AML1/ETO fusion transcript in patients treated with allogeneic bone marrow transplantation for t(8;21) leukemia. Blood 1996;88: 2183–2191.
154 Petz LD, Yam P, Wallace RB, Stock AD, de Lange G, Knowlton RG, Brown VA, Donis-Keller H, Hill LR, Forman SJ, et al: Mixed hematopoietic chimerism following bone marrow transplantation for hematologic malignancies. Blood 1987;70: 1331–1337.
155 Loken MR, Brosnan JM, Bach BA, Ault KA: Establishing optimal lymphocyte gates for immunophenotyping by flow cytometry. Cytometry 1990; 11:453–459.
156 Baumgarth N, Roederer M: A practical approach to multicolor flow cytometry for immunophenotyping. J Immunol Methods 2000;243:77–97.
157 Volk HD, Reinke P, Docke WD: Immunological monitoring of the inflammatory process: Which variables? When to assess? Eur J Surg Suppl 1999: 70–72.
158 Sedlmayr P, Rabinowich H, Elder EM, Ernstoff MS, Kirkwood JM, Herberman RB, Whiteside TL: Depressed ability of patients with melanoma or renal cell carcinoma to generate adherent lymphokine-activated killer cells. J Immunother 1991; 10:336–346.
159 Whiteside TL, Vujanovic NLHerberman RB: Natural killer cells and tumor therapy. Curr Top Microbiol Immunol 1998;230:221–244.
160 Rosenberg SA, Lotze MT, Yang JC, Topalian SL, Chang AE, Schwartzentruber DJ, Aebersold P, Leitman S, Linehan WM, Seipp CA, et al: Prospective randomized trial of high-dose interleukin-2 alone or in conjunction with lymphokine-activated killer cells for the treatment of patients with advanced cancer. J Natl Cancer Inst 1993;85: 622–632.
161 Klingemann HG: Relevance and potential of natural killer cells in stem cell transplantation. Biol Blood Marrow Transplant 2000;6:90–99.
162 Serbina N, Pamer EG: Quantitative studies of CD8+ T-cell responses during microbial infection. Curr Opin Immunol 2003;15:436–442.
163 Hebart H, Daginik S, Stevanovic S, Grigoleit U, Dobler A, Baur M, Rauser G, Sinzger C, Jahn G, Loeffler J, Kanz L, Rammensee HG, Einsele H: Sensitive detection of human cytomegalovirus peptide-specific cytotoxic T-lymphocyte responses by interferon-gamma-enzyme-linked immunospot assay and flow cytometry in healthy individuals and in patients after allogeneic stem cell transplantation. Blood 2002;99:3830–3837
164 Knabel M, Franz TJ, Schiemann M, Wulf A, Villmow B, Schmidt B, Bernhard H, Wagner H, Busch DH: Reversible MHC multimer staining for functional isolation of T-cell populations and effective adoptive transfer. Nat Med 2002;8:631–637.
165 Rauser G, Einsele H, Sinzger C, Wernet D, Kuntz G, Assenmacher M, Campbell JD, Topp MS: Rapid generation of combined CMV-specific CD4+and CD8+ T-cell lines for adoptive transfer into allogeneic stem cell transplant recipients. Blood 2004; 103:3565–3572.
166 Einsele H, Roosnek E, Rufer N, Sinzger C, Riegler S, Loffler J, Grigoleit U, Moris A, Rammensee HG, Kanz L, Kleihauer A, Frank F, Jahn G, Hebart H: Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. Blood 2002;99:3916–3922.
167 Whiteside TL, Zhao Y, Tsukishiro T, Elder EM, Gooding W, Baar J: Enzyme-linked immunospot, cytokine flow cytometry, and tetramers in the detection of T-cell responses to a dendritic cell-based multipeptide vaccine in patients with melanoma. Clin Cancer Res 2003;9:641–649.
168 Ozdemir O, Ravindranath Y, Savasan S: Cell-mediated cytotoxicity evaluation using monoclonal antibody staining for target or effector cells with annexinV/propidium iodide colabeling by fluorosphere-adjusted counts on three-color flow cytometry. Cytometry 2003;56A:53–60.
169 Zarcone D, Tilden AB, Cloud G, Friedman HM, Landay A, Grossi CE: Flow cytometry evaluation of cell-mediated cytotoxicity. J Immunol Methods 1986;94:247–255.
170 de Boer F, Drager AM, Pinedo HM, Kessler FL, van der Wall E, Jonkhoff AR, van der Lelie J, Huijgens PC, Ossenkoppele GJ, Schuurhuis GJ: Extensive early apoptosis in frozen-thawed CD34-positive stem cells decreases threshold doses for haematological recovery after autologous peripheral blood progenitor cell transplantation. Bone Marrow Transplant 2002;29:249–255.
171 Schuurhuis GJ, Muijen MM, Oberink JW, de Boer F, Ossenkoppele GJ, Broxterman HJ: Large populations of non-clonogenic early apoptotic CD34-positive cells are present in frozen-thawed peripheral blood stem cell transplants. Bone Marrow Transplant 2001;27:487–498.
172 Fischer K, Mackensen A: The flow cytometric PKH-26 assay for the determination of T-cell mediated cytotoxic activity. Methods 2003;31:135–142.
173 Fischer K, Andreesen R, Mackensen A: An improved flow cytometric assay for the determination of cytotoxic T lymphocyte activity. J Immunol Methods 2002;259:159–169.
174 Berger TG, Feuerstein B, Strasser E, Hirsch U, Schreiner D, Schuler G, Schuler-Thurner B: Large-scale generation of mature monocyte-derived dendritic cells for clinical application in cell factories. J Immunol Methods 2002;268:131–140.
175 Vuckovic S, Gardiner D, Field K, Chapman GV, Khalil D, Gill D, Marlton P, Taylor K, Wright S, Pinzon-Charry A, Pyke CM, Rodwell R, Hockey RL, Gleeson M, Tepes S, True D, Cotterill A, Hart DN: Monitoring dendritic cells in clinical practice using a new whole blood single-platform TruCOUNT assay. J Immunol Methods 2004;284: 73–87.
176 MacDonald KP, Munster DJ, Clark GJ, Dzionek A, Schmitz J and Hart DN: Characterization of human blood dendritic cell subsets. Blood 2002; 100:4512–4520.
177 Gottfried E, Krieg R, Eichelberg C, Andreesen R, Mackensen A, Krause SW: Characterization of cells prepared by dendritic cell-tumor cell fusion. Cancer Immunol 2002;2:15.