High sensitivity of the Hematoflow™ solution for chronic myelomonocytic leukemia screening

Romain Vazquez, Mikaël Roussel, Bouchra Badaoui, Nicolas Freynet, Sihem Tarfi, Eric Solary, Dorothée Selimoglu-Buet, Orianne Wagner-Ballon

High sensitivity of the Hematoflow™ solution for chronic myelomonocytic leukemia screening

Romain Vazquez[1], Mikael Roussel[2,3], Bouchra Badaoui[1,4], Nicolas Freynet[1], Sihem Tarfi[1,4], Eric Solary[5], Dorothée Selimoglu-Buet[5], Orianne Wagner-Ballon[1,2] on behalf of the Groupe Francophone des Myélodysplasies (GFM)

[1] Hôpitaux universitaires Henri Mondor, APHP, Département d'Hématologie et Immunologie biologiques, F-94100 Créteil, France
[2] CHU Rennes, Laboratoire d'Hématologie, Pôle de Biologie, F-35033 Rennes, France
[3] INSERM, UMR U1236, Université Rennes 1, EFS Bretagne, Equipe Labellisée Ligue Contre le Cancer, F-35043 Rennes, France
[4] Inserm U955, Université Paris-Est F-94100 Créteil, France
[5] Inserm UMR1170, Faculté de Médecine, Université Paris-Sud, Gustave Roussy, 114 rue Edouard Vaillant, F-94805 Villejuif, France

Corresponding author: Prof Orianne Wagner-Ballon
Hôpitaux universitaires Henri Mondor, APHP, Département d'Hématologie et Immunologie biologiques; Inserm U955, Université Paris-Est Créteil ; 51 avenue du Maréchal de Lattre de Tassigny, F-94100 Créteil, France
Phone : + 33 1 49 81 28 80 ; Fax : + 33 1 49 81 28 78 ; email : orianne.wagnerballon@aphp.fr

Article type: Brief Communication

Running title: Hematoflow use for CMML screening

Keywords: chronic myelomonocytic leukemia, reactive monocytosis, classical monocyte accumulation, Hematoflow™, flow cytometry

Total word count: 1061

Abstract word count: 197

Abstract

Background: Accumulation of classical monocytes $CD14^{++}CD16^{-}$ (also called MO1) ≥94% can accurately distinguish chronic myelomonocytic leukemia (CMML) from reactive monocytosis. The HematoFlow™ solution, able to quantify CD16 negative monocytes, could be a useful tool to manage monocytosis which remains a common issue in routine laboratories.

Methods: Classical monocytes were quantified from 153 whole blood samples collected on EDTA using both flow cytometry methods, either MO1 percentage determination by the multiparameter assay previously published and regarded here as the reference method, or CD16 negative monocyte percentage determination by the means of HematoFlow™.

Results: Both methods of classical monocyte percentage determination were highly and significantly correlated ($r=0.87$, $p<0.0001$). The HematoFlow™ solution leant towards an overestimation of the genuine classical monocyte percentages obtained by the reference method. Percentages of CD16 negative monocytes provided by HematoFlow™ were higher than 94% for all the 73 patients displaying classical monocytes MO1 found ≥94% by the reference method, indicating a sensitivity of 100%. Furthermore, the calculation of CD16 negative monocyte percentage can be easily computerized and integrated to the middleware.

Conclusions: We propose a new application of the Hematoflow™ solution that can be used as a flag system for monocytosis management and CMML detection.

Brief communication

Monocytosis management is a very common issue in routine laboratories. We recently demonstrated that a multiparameter flow cytometry assay was very efficient in distinguishing chronic myelomonocytic leukemia (CMML) from reactive monocytosis (1). Circulating monocytes are commonly recognized as three phenotypically different subsets, depending on the expression of two surface markers, CD14 and CD16, which separate classical ($CD14^{++}/CD16^{-}$ also called MO1), intermediate ($CD14^{++}/CD16^{+}$ or MO2) and non-classical ($CD14^{low}/CD16^{+}$ or MO3) monocytes (2). Our flow cytometry assay allows characterizing these three monocyte subsets in whole peripheral blood samples. We noticed that CMML patients displayed an increased percentage of MO1 (CD16 negative) at the expense of MO2 and MO3 (both CD16 positive) monocyte subsets. Accumulation of CD16-negative classical monocytes or MO1 ≥ 94% of total monocytes distinguishes CMML from any type of reactive monocytosis with high specificity (94,1%) and sensitivity (91,9%) (1). The 94% threshold was further validated in subsequent studies (3–5) and is now used as a diagnostic marker of CMML (6).

The HematoFlow™ solution (Beckman-Coulter, Brea, CA) provides white blood cell (WBC) differentials by flow cytometry in many routine laboratories (7). This method includes the cell surface marker CD16 that separates monocytes into positive and negative subsets. Hence, it may easily detect CMML samples, as previously suggested by Roussel and colleagues (see published e-Letter related to (1)). We have now compared these two methods of measurement of the classical CD16 negative MO1 monocyte subset, highlighting a new application of Hematoflow™ solution.

This study was conducted in two routine laboratories (Universitary hospital Henri Mondor of Créteil and Universitary hospital of Rennes) having access to these two cytometric methods. The reference method of MO1 percentage determination in peripheral blood was applied as previously described (1). Briefly, blood samples were stained using the following antibodies: anti-CD45, CD2, CD56, CD24, CD14 and CD16 (all purchased from Beckman-Coulter, Brea, CA) and analyzed with a Navios cytometer (Beckman-Coulter). Since the three monocyte subsets do not express a common surface marker, an exclusion gating strategy was developed so as to eliminate the other mature blood cells and thus, quantify accurately the different monocyte subsets using a biparametric histogram CD14/CD16 (Figures 1A and 1B, left panels).

Thirteen cell populations are easily identified by the HematoFlow™ solution, thanks to an exclusion gating strategy among WBC and an autogating software that allows getting rid of any operator intervention (7). Blood samples are labeled with the CytoDiff™ cocktail that consists of six different antibodies against CD2, CD294 (CRTH2), CD19, CD45, and notably CD36 and CD16. The HematoFlow™ exclusion gating strategy leads to the WBC differential, monocytes “MO” being identified as the CD19-negative, CD45-positive and CD36-positive cell population after exclusion of B cells and neutrophils. Interestingly, the automatic gating software defines $CD36^{low}$ as monocytes, thereby identifying the three monocyte subsets. The anti-CD16 antibody is intended to identify granulocytes. Nevertheless, a biparametric histogram (CD16/SSC, referred as « histogram 12 », Figures 1A and 1B, right panels) conditioned on the “MO” cell population distinguishes CD16-positive “M^+” from CD16-negative “M^-” monocytes. These two features of the Hematoflow™ solution provide a fair approximation of the CD16-negative to CD16-positive monocyte subset fraction,

the percentage of CD16-negative monocytes being easily calculated by dividing the number of CD16-negative monocyte "M^-" events by the number of total monocyte events.

From January 2016 to April 2017, whole blood samples collected on EDTA that were tested for MO1 percentage determination by the reference method (1) were systematically processed with the HematoFlow™ solution in order to quantify CD16 negative monocyte percentage. Thus, 153 samples were tested for both methods in the same time regardless of the absolute monocyte count and even though a precise diagnosis was not available. It is worth mentioning that no sample was excluded owing to an inaccurate gating of the $CD36^+$ monocytes.

The median of age of the patients was 74 years (range: 15 to 99). Complete blood counts presented wide range of both leukocytosis (median: 8.2 $x10^9$/L; range: 1.7 to 44.1) and absolute monocyte count (median: 1.3 $x10^9$/L; range: 0.1 to 13.9).

Comparison of the fraction of classical monocytes (MO1) measured by using the reference method and the fraction of CD16-negative monocytes calculated by the HematoFlow™ showed a significant correlation between the two methods (r^2=0.75; slope=0.7540; $p<0.0001$) (Figure 1C). Interestingly, measurement of the CD16-negative monocyte percentages obtained with HematoFlow™ leant towards a slight overestimation of the genuine classical monocyte percentages obtained by the reference method (1) (Figure 1D, Bland-Altman plot).

Of the 73 cases with classical monocytes MO1 ≥94% by the reference method (i.e. suspected of being diagnosed as CMML (1,4)), all were found having a CD16-negative monocyte percentage ≥ 94% by the Hematoflow™ method (Figure 1E). In other words, the Hematoflow™ solution did not generate any false negative result,

indicating a sensitivity of 100%. Conversely, of the 80 patients with a fraction of classical monocytes MO1 <94% by the reference method (i.e. not being diagnosed as a CMML according to this parameter(1)), 22 (27.5%) displayed a percentage of CD16-negative monocytes ≥94%, indicating a specificity of 72.5%.

Accumulation of classical monocytes ≥94% is now recognized as a powerful tool to diagnose CMML (1,3–5). This relative accumulation of classical monocytes in CMML patients seems to be due to the disappearance of non classical monocytes, the precise mechanism of this maturation blockage being still unexplained. The HematoFlow™ solution provides a useful approximation of the MO1 percentage obtained with the multiparameter flow cytometry assay described in (1). We suggest that the high sensitivity of the HematoFlow™ solution, which leads to an overestimation of classical monocyte percentage, positions this solution as a flag system for monocytosis management. This new application makes the Hematoflow™ solution an exciting tool for screening rapidly and accurately the pathological monocytosis at no extra cost. Importantly, the calculation of the CD16-negative monocyte percentage can be easily computerized and integrated to the middleware without needing an operator intervention. Thus, as the HematoFlow™ differential is available for many CBC, detection of a monocytosis combined with the measurement of the CD16 negative monocyte fraction obtained at the very same time, may improve our management of monocytosis, an extremely frequent issue in routine laboratories, by deciphering which samples need further flow cytometry investigation by the reference method (1).

We report here a new application of the Hematoflow™ solution, which may be helpful to all the laboratories that have implemented this flow cytometry differential method in managing daily monocytosis and detecting those that could indicate a CMML.

Conflict of interest

Prof Solary, Prof Wagner-Ballon and Dr Selimoglu-Buet have a patent issued, relevant to the work. The other authors declare no conflict of interest.

Author contribution

R.V., M.R., B.B., N.F. and S.T. performed experiments and analyzed data.

E.S., D.S-B. and O.W-B. conceived the study, analyzed data and wrote the paper.

All authors revised and approved the manuscript.

Literature cited

1. Selimoglu-Buet D, Wagner-Ballon O, Saada V, Bardet V, Itzykson R, Bencheikh L, Morabito M, Met E, Debord C, Benayoun E, Nloga A-M, Fenaux P, Braun T, Willekens C, Quesnel B, Adès L, Fontenay M, Rameau P, Droin N, Koscielny S, Solary E, Francophone Myelodysplasia Group. Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia. Blood 2015;125:3618–3626.

2. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJM, Liu Y-J, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM, Lutz MB. Nomenclature of monocytes and dendritic cells in blood. Blood 2010;116:e74-80.

3. Talati C, Zhang L, Shaheen G, Kuykendall A, Ball M, Zhang Q, Lancet JE, Zuckerman KS, List AF, Komrokji R, Moscinski L, Padron E. Monocyte subset analysis accurately distinguishes CMML from MDS and is associated with a favorable MDS prognosis. Blood 2017;129:1881–1883..

4. Selimoglu-Buet D, Badaoui B, Benayoun E, Toma A, Fenaux P, Quesnel B, Etienne G, Braun T, Abermil N, Morabito M, Droin N, Solary E, Wagner-Ballon O. Accumulation of classical monocytes defines a subgroup of MDS that frequently evolve into CMML. Blood 2017;130:832–835.

5. Patnaik MM, Timm MM, Vallapureddy R, Lasho TL, Ketterling RP, Gangat N, Shi M, Tefferi A, Solary E, Reichard KK, Jevremovic D. Flow cytometry based monocyte subset analysis accurately distinguishes chronic myelomonocytic leukemia from myeloproliferative neoplasms with associated monocytosis. Blood Cancer J. 2017;7:e584.

6. Ball M, List AF, Padron E. When clinical heterogeneity exceeds genetic heterogeneity: thinking outside the genomic box in chronic myelomonocytic leukemia. Blood 2016;128:2381–2387.

7. Roussel M, Benard C, Ly-Sunnaram B, Fest T. Refining the white blood cell differential: the first flow cytometry routine application. Cytom. Part J. Int. Soc. Anal. Cytol. 2010;77:552–563.

8. Bardet V, Wagner-Ballon O, Guy J, Morvan C, Debord C, Trimoreau F, Benayoun E, Chapuis N, Freynet N, Rossi C, Mathis S, Gourin M-P, Toma A, Béné MC, Feuillard J, Guérin E, Groupe Francophone des Myélodysplasies (GFM), Groupe d'Etude Immunologique des Leucémies (GEIL). Multicentric study underlining the interest of adding CD5, CD7 and CD56 expression assessment to the flow cytometric Ogata score in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Haematologica 2015;100:472–478.

9. Cottard A, Wagner-Ballon O, Le Priol J, Azzaoui I, Ly-Sunarram B, Fest T, Roussel M. Improvement of the leukocyte differential performed by flow cytometry using the

advanced 2.0 version of the CytoDiff CXP software. Cytom. Part J. Int. Soc. Anal. Cytol. 2014;85:653–657.

Figure legend

Figure 1. Classical monocyte percentage determination by the Hematoflow™ solution compared with the reference method established by Selimoglu-Buet *et al* (1).

A-B: Representative histograms used for classical monocyte percentage determination by both the reference method (MO1 in bright green defined as $CD14^{++}CD16^{-}$ on CD14 vs CD16 histogram, left panels) and the Hematoflow™ solution (CD16-negative monocytes in bright green defined as $CD16^{-}$ on CD16 vs SSC histogram, right panels) for a patient displaying classical monocytes <94% (A) or classical monocytes ≥94% (B). **C-D:** Correlation (C) and bias (shown by Bland and Altman plot, D) between the percentage of MO1 obtained by the reference method and the percentage of CD16-negative monocytes obtained by the Hematoflow™ solution on 153 peripheral blood samples. *All percentages (%) were compared using a Pearson correlation test (determination factor r^2 is shown); MedCalc Statistical Software version 17.6 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2017). Each dot represents one sample.* **E:** CD16-negative monocyte percentages obtained by the Hematoflow™ solution for patients separated in two groups according to the reference method, displaying either classical monocytes MO1 ≥94% (blue dots) or MO1 <94% (orange dots).

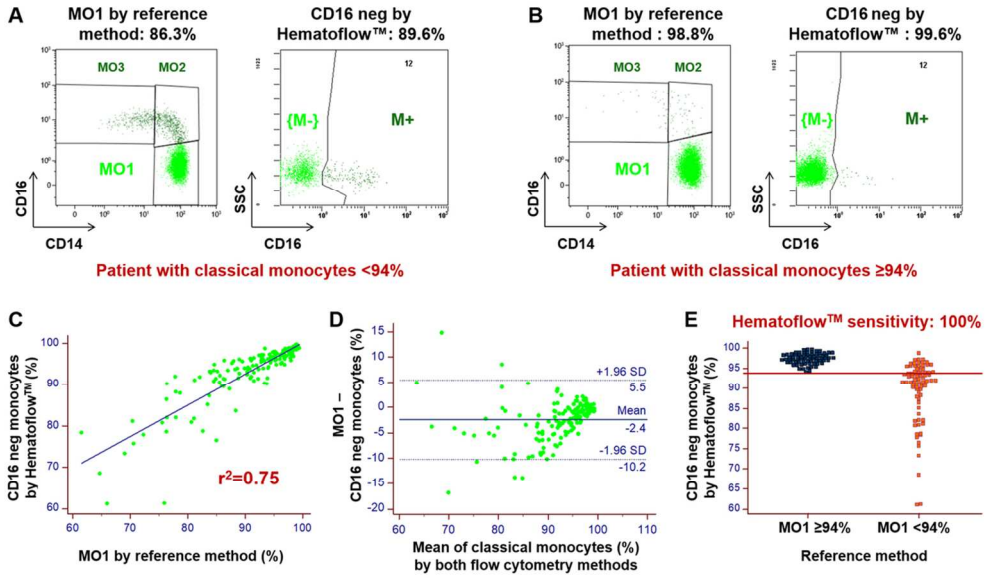

Figure 1

110x64mm (300 x 300 DPI)