European Journal of Cancer 54 (2016) 49–56

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejcancer.com

Original Research

# Multiparameter flow cytometry is instrumental to distinguish myelodysplastic syndromes from non-neoplastic cytopenias

Eline M.P. Cremers [a], Theresia M. Westers [a], Canan Alhan [a], Claudia Cali [a], Mariëlle J. Wondergem [a], Pino J. Poddighe [b], Gert J. Ossenkoppele [a], Arjan A. van de Loosdrecht [a,*]

[a] *Department of Hematology, VU University Medical Center, Cancer Center Amsterdam (VUmc CCA), De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands*
[b] *Department of Clinical Genetics, VU University Medical Center, Cancer Center Amsterdam (VUmc CCA), De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands*

Received 31 March 2015; received in revised form 2 July 2015; accepted 10 November 2015
Available online xxx

**KEYWORDS**
Myelodysplastic syndromes;
Flow cytometry;
Cytopenias;
Diagnosis

**Abstract** Mandatory for the diagnosis of myelodysplastic syndromes (MDS) is the presence of dysplasia in >10% of cells within one or more cell lineages or presence of >15% ring sideroblasts or presence of MDS-associated cytogenetic (CG) abnormalities. Discrimination between neo-plastic and non-neoplastic causes of cytopenias can be challenging when dysplastic features by cytomorphology (CM) are minimal and CG abnormalities are absent or non-discriminating from other myeloid neoplastic disorders. This study evaluated a standard diagnostic approach in 379 patients with unexplained cytopenias and highlights the additional value of flow cytometry (FC) in patients with indeterminate CM and CG. CM reached no clear-cut diagnosis in 44% of the patients. Here, CG was able to identify two additional patients with MDS; other CG results did not reveal abnormalities or were not contributory. Based on the FC results, patients without a diagnosis by CM and CG were categorized 'no MDS-related features' (65%), 'limited number of MDS-related changes' (24%), and 'consistent with MDS' (11%). Patients were followed over time in an attempt to establish or confirm a diagnosis (median follow-up 391 d, range 20–1764). The specificity (true negative) of MDS-FC analysis calculated after follow-up was 95%. FC can aid as a valuable tool to exclude

* *Corresponding author:* Department of Hematology, VU Medisch Centrum, Cancer Center Amsterdam; De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands. Tel.: +31 20 4442604.
*E-mail addresses:* e.cremers@vumc.nl (E.M.P. Cremers), tm.westers@vumc.nl (T.M. Westers), c.alhan@vumc.nl (C. Alhan), c.cali@vumc.nl (C. Cali), m.wondergem@vumc.nl (M.J. Wondergem), p.poddighe@vumc.nl (P.J. Poddighe), g.ossenkoppele@vumc.nl (G.J. Ossenkoppele), a.vandeloosdrecht@vumc.nl (A.A. van de Loosdrecht).

http://dx.doi.org/10.1016/j.ejca.2015.11.013

MDS when CM and additional CG are not conclusive in patients with cytopenia.


## 1. Introduction

Myelodysplastic syndromes (MDS) constitute a heterogeneous group of haematopoietic stem cell disorders [1]. For the diagnosis of MDS, cytomorphological (CM) assessment of peripheral blood and bone marrow (BM) in combination with cytogenetic (CG) analysis are mandatory [2–4]. The World Health Organisation (WHO) classification is based on CM and CG findings in blood and BM evaluating cytopenias, dysplasia, presence of ring sideroblasts, enumeration of blasts, Auer rods and CG findings [3,4]. The minimal CM criterion for MDS is the presence of dysplasia in >10% of the cells in at least one cell lineage. This is challenging since dysplasia can also occur in myeloid neoplasms other than MDS or in reactive conditions. Therefore, other possible causes of dysplasia such as deficiencies or viral infections should be excluded before the diagnosis of MDS can be established [5,6]. A supplementary hallmark in the diagnosis is the presence of MDS-associated CG abnormalities, present in approximately 40–50% of the patients [7,8]. However, abnormalities described as typical MDS are commonly seen in other myeloid neoplasms [9,10]. For example, a trisomy of chromosome 8 does not lead to the diagnosis of MDS, as this abnormality can also occur in myeloid proliferative disorders. Therefore, CG results should always be interpreted in the context of CM results. In the cases of minimal abnormalities by CM and absence of (MDS-associated) CG abnormalities, there is a need for additional diagnostic tools. Flow cytometry (FC) has proven additional value in the identification of myelodysplasia [11,12]. The aim of the present study is to evaluate the use of FC as a diagnostic tool when CM and CG are not informative in a cohort of patients with unexplained cytopenias.

## 2. Patients and methods

### 2.1. Patients

Bone marrow samples of 379 adult patients with cytopenias, according to WHO criteria, sent to our department from January 2009 to January 2014 were evaluated. Median age was 66 years (range 20–94), 245 males and 134 females (Table 1). In accordance with the diagnostic guidelines, CM assessment by at least one experienced haematologist–cytologist was performed [11,13]. The haematologist–cytologist determined the necessity of CG and molecular biology (MB). FC was performed standardly (Supplementary data). Patients were followed over time until a diagnosis was established. The study was approved by the local ethical committee and in accordance with the declaration of Helsinki.

### 2.2. Flow cytometry

Data generated by FC were used to calculate validated MDS-FC scores: the diagnostic score and the (modified) flow cytometric scoring system (FCSS), respectively [14–16]. Both scores were integrated into one FC result [17].

#### 2.2.1. Diagnostic score

This four-parameter diagnostic score comprises the percentage of $CD34^+$-myeloid progenitors in nucleated cells, percentage of B-cell progenitors within $CD34^+$ compartment, CD45 expression level of $CD34^+$-myeloid progenitors, and sideward light scatter (SSC) peak channel value of granulocytes. Each abnormality (compared to reference ranges) scores 1 point; ≥2 points allocates 'MDS' [14].

#### 2.2.2. Flow cytometric scoring system

The FCSS evaluates differences from normal regarding percentages, expression levels, maturation patterns and aberrant expression levels of lineage-specific and lineage infidelity markers of immature myeloid progenitor cells and maturing granulocytes and monocytes. Patients were categorized 'no-mild dysplasia' (0–1 points), 'moderate dysplasia' (2–3 points), and 'severe dysplasia' (≥4 points). Cutler et al. modified this score by adding an extra point in case of <5% abnormal progenitors without additional aberrancies [15,16].

#### 2.2.3. Integrated flow cytometric score

The integrated flow cytometric score (iFS) combines the diagnostic score and the modified FCSS into one FC result (Table 2). The diagnostic score separates patients into two categories: '<2 points' versus '≥2 points'. Second is the evaluation of the myeloid progenitor cells (SSC low intermediate/$CD34^+$ and/or $CD117^+$) separating patients into two categories: normal or aberrant myeloid progenitors. Third is the evaluation of neutrophils and monocytes according to parameters described in European LeukemiaNet (ELN) guidelines, separating patients into normal or aberrant myelopoiesis [18]. According to this strategy, patients were classified as 'no MDS-related features', 'limited number of MDS-related changes', or 'consistent with MDS'.