Leukemia Research 79 (2019) 69–74

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Flow cytometry diagnosis in myelodysplastic syndrome: Current practice in Latin America and comparison with other regions of the world

S. Grille[a,b,1], M. Iastrebner[c,1], I. Lorand-Metze[d,1], V.H.J. van der Velden[e], M.R.V. Ikoma[f,1], G. Vidal-Senmache[g,1], E. Colado[h], L.J. Rabelo-Carrasco[i,1], A. Blanco[j], F. Huamán-Garaicoa[k,1], J.E. O'Connor[l], C. Cao Pochintesta[m,1], E. Jensen[n,1], N.S. Bacal[o,1], L. Díaz[b,1], D. Lens[a,*,1]

[a] Laboratorio de Citometria y Biologia Molecular, Departamento Basico de Medicina, Hospital de Clínicas, Facultad de Medicina, Universidad de la Republica, Montevideo, Uruguay
[b] Catedra de Hematologia, Hospital de Clinicas, Universidad de la Republica, Montevideo, Uruguay
[c] Servicio de Hematologia, Sanatorio "Sagrado Corazón", Buenos Aires, Argentina
[d] Hematology - Hemotherapy Center, University of Campinas, Campinas, São Paulo, Brazil
[e] Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Dutch Cytometry Society, the Netherlands
[f] Laboratory of Cytometry, Hospital Amaral Carvalho, Jaú, São Paulo, Brazil
[g] Hospital Edgardo Rebagliati Martins, Lima, Peru
[h] Hematology and Laboratory Medicine Department and Instituto Universitario de Oncología del Principado de Asturias, Central University Hospital of Asturias, Oviedo, Spain
[i] Hospital de Especialidades. Centro Médico Nacional Siglo XXI- IMSS, Ciudad de México, Mexico
[j] Flow Cytometry Core Technologies, UCD Conway Institute, University College Dublin, Dublin, Ireland
[k] Universidad Catolica de Santiago de Guayaquil, Instituto Oncologico Nacional SOLCA, Ecuador
[l] Laboratory of Cytomics, Joint Research Unit CIPF-UVEG, Department of Biochemistry and Molecular Biology, University of Valencia, Valencia, Spain
[m] Clinica Universidad de Los Andes y Laboratorio Citometría de Redsalud, Santiago de Chile, Chile
[n] Hospital Nacional de Niños, San José, Costa Rica
[o] Clinical Pathology Laboratory, Division of Hematology and Flow Cytometry, Hospital Israelita Albert Einstein, São Paulo, Brazil

ARTICLE INFO

Keywords:
Myelodysplastic syndrome
Flow cytometry
Survey
Diagnosis

ABSTRACT

Background: Flow cytometry (FC) is a valuable tool for the diagnosis of myelodysplastic syndromes (MDS). We present results of a survey carried out to evaluate FC current practice for MDS diagnosis in Latin America (LA), focusing on markers used and characteristics of the clinical diagnostic report. Compliance to IMDSflow recommendations was also evaluated. These practices were then compared with those used in other countries.
Methods: An online survey was sent through the Grupo Latino-Americano de Mielodisplasia to LA cytometrists and other international scientific societies.
Results: 91 responses from 15 LA countries were received. The median of the number of markers used was 20 ± 4.5, but only 8.1% of participants adopted the complete panel proposed by the International/European LeukemiaNet Working Group (IMDSflow). We received 140 eligible answers from regions other than LA (66 Europe, 59 USA-Canada, 8 Oceania, 6 Asia and 1 Africa). LA utilized more markers for MDS diagnosis than USA/Canada ($p = 0.006$), but similar to Europe. The use of MDS scoring systems differed among regions: 10.3% in LA, 0% USA/Canada and 25.7% Europe reported the "Ogata score". Finally, 52.0% of all participants included a general interpretation statement in the final report about the consistency of the FC results with MDS diagnosis, with no statistical differences between regions.
Conclusions: This survey shows a low compliance with the IMDSflow recommendations and a scarce use of the scoring systems proposed in the literature. However, the number of surface markers used is high. We will work to develop a FC consensus for MDS diagnosis adapted to the clinical practice requirements in LA.

* Corresponding author at: Laboratorio de Citometria y Biologia Molecular, Departamento Basico de Medicina, Hospital de Clinicas, Universidad de la Republica, Montevideo, Uruguay. Tel.: +598 99872374.
E-mail addresses: daniela.lens@gmail.com, lab.citometría.hc@gmail.com (D. Lens).
[1] On behalf of the Grupo Latinoamericano de Síndromes Mielodisplásicos (GLAM).

https://doi.org/10.1016/j.leukres.2019.01.009
Received 10 December 2018; Received in revised form 19 January 2019; Accepted 21 January 2019
Available online 24 January 2019

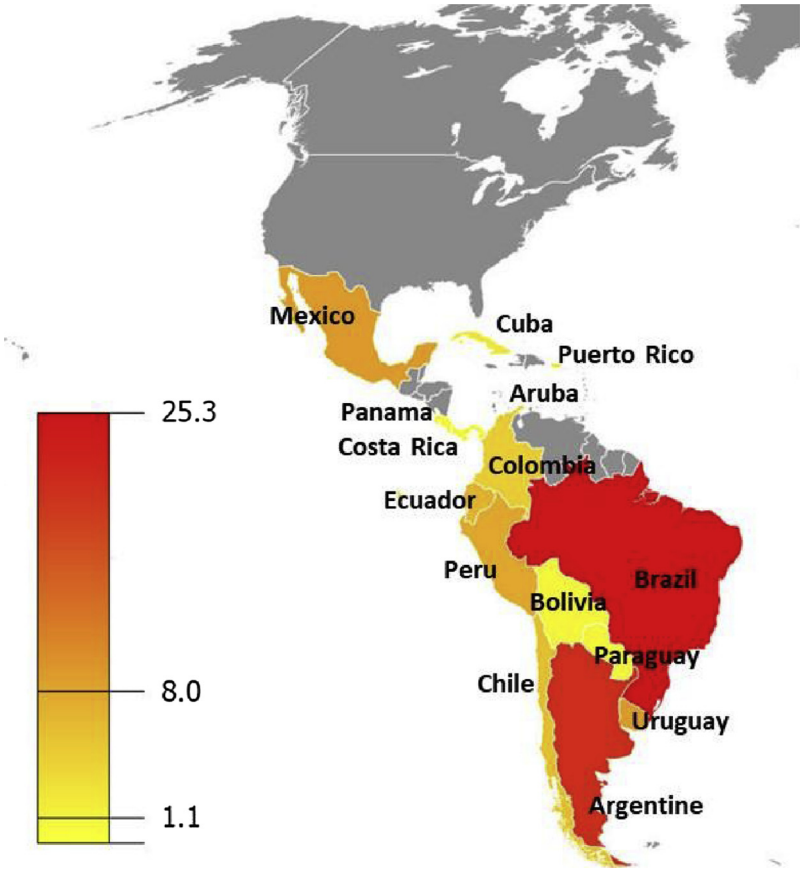

Fig. 1. Geographic distribution of LA respondents. Yellow denotes a lower number of participants/country while red represents a higher number of participants/country (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

1. Introduction

Myelodysplastic syndromes (MDS) are an heterogeneous group of clonal hematopoietic disorders characterized by dysplasia, bone marrow (BM) failure and an increased risk of acute myeloid leukemia [1]. MDS diagnosis is challenging, especially in the absence of cytogenetic abnormalities, BM blasts less than 5% or ring sideroblasts less than 15%. Many methods used to diagnose MDS patients, particularly cytogenetics and morphological BM assessment, depend on individual experience, and therefore, efforts should be directed to identify novel diagnostic tools to make MDS diagnosis more accurate [2].

Multiparametric flow cytometry (FC) allows identification of specific aberrations in both the immature and mature compartments among bone marrow hematopoietic cell lineages (erythroid, myeloid and monocytic) contributing to MDS diagnosis [3]. Although the WHO 2016 classification of hematopoietic neoplasms does not recognize FC findings alone as sufficient to establish a primary diagnosis of MDS in the absence of morphological and/or cytogenetic findings, it has introduced FC as an important co-criterion in MDS, particularly in patients with inconclusive morphology and/or cytogenetics [4,5]. Current recommendations support the use of FC as a valuable additional diagnostic tool for MDS [6–10]. Particularly the guidelines of the International/European LeukemiaNet Working Group for Flow Cytometry in MDS (IMDSflow) recommend antibody panels, and provide technical recommendations to harmonize the use of FC in the diagnosis of MDS [7,8].

Here we present the work carried out by the Myelodysplastic Syndromes Latin-American Group (GLAM) for evaluation of FC current practice for MDS diagnosis in Latin America (LA), focusing on the immunophenotypic markers studied and information contained in the final clinical diagnostic report. Thus, we assessed the compliance to IMDSflow recommendations. In addition, we compared these practices with those used in other regions of the world.

2. Methods

A 5-question survey (SurveyMonkey® Inc.; Palo Alto, California, USA) was electronically distributed to members of GLAM and flow cytometry national societies in LA. In order to compare LA results with other countries, the survey was translated to English and distributed among members of the European Society for Clinical Cell Analysis (ESCCA), the International Society for Advancement of Cytometry (ISAC), the International Clinical Cytometry Society (ICCS), Dutch Cytometry Society, the Iberian Cytometry Society (SIC), as well as sent to the Purdue University Cytometry mailing list. Responses were collected during an eleven-month period (November 2016 - September 2017) (Supplementary Figure s1).

The survey contained relevant questions regarding country of origin, number of fluorescence colors used in the panel, number of MDS samples received per month, markers used for MDS diagnosis and data included in the report (Supplementary Figure s1).

Participation was voluntary and permission to publish the results was requested. All data collected were anonymous, so the responding centers could not be identified except for country of origin.

2.1. Statistical analysis

Categorical variables were expressed as frequencies and percentages and quantitative variables as median and interquartile range (IQR). For categorical variables the statistical significance of differences was evaluated using Chi-Square test and for continuous variables using Mann-Whitney test. A value of $p < 0.05$ was considered statistically

Table 1
Characteristics of laboratories participants.

		Europe	Latin America	USA and Canada	p value
Flow cytometers features	3 colors	0*	3 (3.4%)	0	0.012
	4 colors	4 (6.1%)*	13 (14.9%)	8 (13.8%)	
	5 colors	0*	1 (1.1%)	4 (6.9%)	
	6 colors	4 (6.1%)*	14 (16.1%)	13 (22.4%)	
	8 colors	47 (71.2%)*	48 (55.2%)	17 (29.3%)	
	more than 8 colors	11 (16.7%)*	8 (9.2%)	16 (27.6%)	
Number of samples received per month.	less than 5	6 (9.1%)*	29 (33.3%)	14 (23.7%)	0.009
	5 to 10	27 (40.9%)*	27 (31%)	20 (33.9%)	
	more than 10	33 (50%)*	31 (35.6%)	25 (42.4%)	

Data shown are absolute frequency and percentage.
* $p < 0.05$ compared to Latin America.

significant. Analyses were performed using SPSS 21.0 software.

3. Results

3.1. Latin America survey results

To test the current practice in LA of FC for MDS diagnosis a 5-question electronic survey was conducted (Supplementary Fig. s1). 91 responses to the survey were obtained from 15 L A countries. 4 responses were excluded from the analysis, one because it did not perform FC analysis of MDS samples and 3 because they did not consent to publish their data. Fig. 1 shows the country of origin of the 87 eligible participants from LA (Supplementary Table 1s). As shown in Table 1, 64.4% of LA laboratories used flow cytometers with 8 or more colors. The number of samples for MDS diagnosis received monthly was: 33.3% of the centers receive less than 5 samples, 31% receive from 5 to 10 and 35.6% receive more than 10 (Table 1).

3.2. Immunophenotypic markers used for the analysis of dysplasia by FC

Queries regarding the number of markers used showed a median of 20 markers (IQR, 16–24) (Table 2). The majority of the participants (n = 80, 92%) use CD45, CD34, CD117 and HLA-DR antibodies for identification and quantification of bone marrow progenitor cells (Table 2). The most common markers used to gate the progenitor compartment were: CD45, CD34 and CD117 and to analyze neutrophil maturation patterns CD13, CD11b and CD16. 70 participants (80.5%) include all these 6 markers.

Table 3 shows the proposed core markers for FC dysplasia analysis suggested by the IMDSflow [8,9]. We analyzed the compliance of the participants to this guideline. The IMDSflow recommends 20 general markers for the complete study of bone marrow dysplasia [8,9]. Only 7 of the participants (8.1%) use all of them, (Table 4). For identification of aberrancies in progenitor cells, only 17 of the participants (19.8%) use all the markers proposed by IMDSflow (Tables 3 and 4). A decrease in B-cell progenitor cells is frequently observed in MDS [11–13]. These cells can be identified with different strategies: $CD34^+$ $CD45^{dim/low}$ SSC^{low}, $CD34^+$ $CD19^+$ or $CD34^+$ $CD10^+$ [14]. 81 participants (93.1%) combine CD34 and CD45 with CD19 and/or CD10 for this purpose (Table 2). Compliance with others IMDSflow recommendations is shown in Table 4.

Although monocytes can be identified and quantified on SSC/CD45 bivariate plot, additional monocyte markers such as CD14, CD64, CD36 or CD33 improve the selection of this population. The use of CD14 alone may underestimate the percentage of monocytic cells, particularly when immature forms are present [9]. All participants used CD14 associated with at least one of the following monocytic markers: CD35, CD36, CD64, IREM-2 (CD300e) or CD33. The IMDSflow recommendations do not include CD35 or CD300e, but 37 (43.0%) and 48 (55.8%) participants include them in their panel, respectively (data not shown). The utility of these markers, together with CD64 and CD14, has already been demonstrated for the evaluation of aberrations in monocytic maturation [15].

Similarly, for identification of erythroid compartment aberrancies, the IMDSflow recommendations do not include CD105 but 47 participants (54.6%) use it (data not shown).

Megakaryocytic linage analysis has little relevance in FC MDS diagnosis and the IMDSflow recommendations do not include

Table 2
Markers, scores systems and report characteristics used among regions.

	Europe	Latin America	USA/Canada	p value
Markers used				
Number of markers used for MDS study	20.5 (16-24)	21.5 (17-26)	18 (14-21)*	0.006
Markers used for inmature compartment analysis (CD45, CD34, CD117, HLA-DR)	62 (93.9%)	80 (92%)	49 (83.1%)	NS
CD38	29 (43.9%)	44 (51.5%)	4 (6.8%)*	0.0001
TdT	18 (27.3%)	22 (25.3%)	8 (13.6%)	NS
Identification of B progenitor cells with CD10 and/or CD19	60 (90.9%)	81 (93.1%)	53 (89.8%)	NS
Megakaryocytic markers used (CD61 and/or CD42a)	12 (18.2%)	23 (26.4%)	11 (18.6%)	NS
Score system used				
Wells FCSS Score reported	5 (7.6%)	5 (5.7%)	0	0.26
Ogata Score reported	17 (25.8%)*	9 (10.3%)	0*	0.001
Red Score reported	4 (6.1%)*	0	0	0.002
Items included in the report				
Percentage of myeloid progenitor cells	59 (89.4%)	77 (88.5%)	44 (74.6%)	NS
Description of aberrancies in myeloid progenitor cells	57 (86.4%)	71 (81.6%)	37 (62.7%)*	0.011
Percentage of lymphoid progenitor cells	40 (60.6%)	56 (64.4%)	30 (50.8%)	NS
Description of aberrancies identified in mature compartments	53 (80.3%)	76 (87.4%)*	39 (66.1%)*	0.0028
Interpretation if the findings could be consistent with MDS	50 (75.8%)*	42 (48.3%)	19 (32.2%)*	0.001

Data shown are absolute frequency and percentage. NS: not significant.
* $p < 0.05$ compared to Latin America.

Table 3
Proposed markers for flow cytometry dysplasia analysis by European Leukemia Net Working Group Guidelines.

General Core markers	CD45, CD34, CD117, HLA-DR, CD11b, CD13, CD16, CD33, CD14, CD7, CD56, CD19
Erythroid	CD45, CD71, CD235a, CD117, CD36
Progenitors	CD45, CD34, CD117, HLA-DR, CD11b, CD13, CD7, CD56, CD19, CD5, CD15
Maturing neutrophils	CD45, CD34, CD117, HLA-DR, CD11b, CD13, CD16, CD33, CD14, CD64, CD56, CD15, CD10
Monocytes	CD45, CD34, CD117, HLA-DR, CD11b, CD13, CD16, CD33, CD14, CD36, CD64, CD56, CD2

Table 4
Compliance with European Leukemia Net Working Group Guidelines.

	Europe	Latin America	USA and Canada	p value
Complete proposed panel	1 (1.5%)	(7) 8.1%	4 (6.8%)	NS
General core markers	37 (56.1%)	46 (53.5%)	20 (33.9%)	NS
Markers to study aberrancies in progenitor compartment	9 (13.6%)	17 (19.8%)	14 (23.7%)	NS
Markers to study erythroid compartment	16 (24.2%)	22 (25.6%)	6 (10.2%)	NS
Markers to study neutrophils maturing	24 (36.4%)	44 (51.2%)	20 (33.9%)	NS
Markers to study monocyte compartment	14 (21.2%)	18 (20.9%)	11 (18.6%)	NS

megakaryocytic markers [9]. However, 23 participants (26.5%) used CD61 and/or CD42a.

3.3. Scoring system for MDS diagnosis

Several scoring systems have been published to provide useful information for MDS diagnosis. The first was developed by Wells et al. in 2003 [16]. We found that only 14 (16.1%) participants use the markers necessary to calculate Wells score and 5 (5.7%) include it in their final report (Table 2).

Another useful and validated scoring system is the "Ogata score", developed as a screening diagnostic test. This score includes 4 parameters: percentage of CD34+ myeloid progenitor cells in total nucleated cells, frequency of B-cell related precursors within the CD34+ subset, CD45 expression on myeloid progenitors in comparison to lymphocytes and side scatter of granulocytes in comparison to lymphocytes [13]. Although 84 participants (96.6%) include all parameters for "Ogata score" calculation, only 9 (10.3%) report this score (Table 2).

A novel tool for evaluation of erythroid lineage is the "Red score", published by Mathis et al. in 2013 [17]. This score takes into account the following three parameters: coefficient of variance of CD36 and CD71 on CD36+ CD64− CD71+ erythroblasts and the hemoglobin value. None of the LA laboratories include the score in the final report (Table 2).

3.4. Information included in the report

The IMDSflow suggests that FC reports should state whether the findings could be consistent or not with MDS [7]. The information included in the report was: 77 (88.5%) of the responders include the percentage of myeloid progenitor cells, 71 (81.6%) include aberrancies observed on myeloid progenitor cells, 56 (64.4%) report the percentage of lymphoid progenitor cells and 76 (87.4%) describe the aberrancies identified in mature myeloid compartment (monocytic, granulocytic and erythroid) (Table 3). Finally, 42 (48.3%) participants included in the report a general interpretative statement about the consistency of the FC results with MDS diagnosis (Table 2).

3.5. Comparison of LA results with other regions

A total of 174 responses from regions outside LA were received, but 34 were excluded from the analysis because they did not perform FC for MDS and/or because they did not consent to publish their data. Countries of origin of the 140 eligible responses are shown in Supplementary Table 1s. Overall, we received 66 responses from Europe, 59 from USA-Canada, 8 from Oceania (Australia), 6 from Asia and 1 from Africa. Taking into account the low number of responses from Oceania, Asia and Africa, we decided to compare LA results only with Europe and USA-Canada.

As shown in Table 1, Europe has a wider availability for 8 or more color cytometers than LA ($p = 0.0001$), but there is no statistically significant difference between LA and USA/Canada. Additionally, European laboratories receive more samples per month for MDS diagnosis than LA ($p = 0.009$). No statistical differences were found between LA and USA/Canada. Participants from LA use more immunophenotypic markers for MDS diagnosis (Median: 22; IQR 17–26) than USA/Canada (Median: 18; IQR 14–21) ($p = 0.006$), but similar to Europe (Median: 21; IQR 16–24) (Table 1).

The number of markers used for identification of different bone marrow populations, and compliance with IMDSflow Working Group Guidelines was similar between LA, USA/Canada and Europe (Tables 2 and 4).

CD35 and IREM2 (CD300e), markers for identification of monocytes, are not included in the IMDSflow recommendations. CD35 is used by 37 (43.0%) participants in LA, 0% in USA/Canada and 24 (36.3%) in Europe. Similar results were found with CD300e: LA 48 (55.8%), USA/Canada 0% and Europe 29 (43.9%). A statically significant difference was found for CD35 ($p = 0.0001$) and IREM-2 ($p = 0.0001$) use between LA and USA/Canada. Similarly, for detecting erythroid compartment aberrancies, CD105 is used by 47 (54.6%) participants in LA, 1 (16.9%) in USA/Canada and 31 (46.9%) in Europe. LA used CD105 more frequently than USA/Canada ($p = 0.0001$) (Table 2).

As shown in Table 2, the usage of MDS scoring systems differs between regions. Only 9 (10.3%) responders in LA, 0% in USA/Canada and 17 (25.7%) in Europe reported the "Ogata score". LA uses the "Ogata score" more frequently than USA/Canada ($p = 0.011$), but less frequently than Europe ($p = 0.012$). The "Red score" is only reported by European participants ($n = 4$ (1.8%)) (Table 1).

The degree of information included in the report differs between regions (Table 2). The description of aberrancies on myeloid progenitor cells is more frequently included in the report in LA than in USA/Canada ($p = 0.011$) and no statistical difference was observed between LA and Europe. Similar results were obtained for information contained in the report regarding aberrancies identified in the mature myeloid compartment (monocytic, granulocytic and erythroid), between LA and USA/Canada ($p = 0.028$). Finally, an interpretation of compatibility of FC with a diagnosis of MDS is reported by 118 (52.0%) of all participants. This interpretation is less frequent in LA than in Europe ($p = 0.001$), but more frequent than in USA/Canada ($p = 0.049$) (Table 2).

4. Discussion

Currently, the diagnosis of MDS is based on clinical, cytology, histology and cytogenetic data. Although morphologic dysplasia represents a major diagnostic criterion of MDS, it is often difficult to define the degree and quantify due to high inter-observer variability [18]. Cytogenetics is currently considered the most important parameter because the karyotype has diagnostic, prognostic and therapeutic implications.. However, the combination of bone marrow dysplasia and clonal cytogenetic abnormalities is only found in some patients, usually those with advanced disease. Approximately half of the patients have a normal karyotype and MDS diagnosis is based exclusively on the morphological evaluation [5,20]. In those cases, the diagnosis of MDS is challenging, and additional confirmatory tools are needed. In the near future, Next Generation Sequencing will become more broadly available in our countries and will allow the demonstration of the presence of clonal hematopoiesis in a greater proportion of MDS patients with profound implications for prognosis and treatment decisions" [19]

Although immunophenotypic abnormalities such as the presence of three or more aberrancies in at least two cell compartments have been associated with MDS, they are not diagnostic in the absence of conclusive morphological and/or cytogenetic criteria [21,22]. As there are no antigenic hallmarks associated with dysplasia, FC therefore relies most commonly on a pattern recognition-based approach, which is an inherent weakness of FC in MDS diagnosis.

FC data remains limited in several steps of the process of MDS diagnosis due to the lack of standardization in processing and handling of samples, antibody clones, antibody combination panels, fluorochromes, cytometer settings and analysis strategies. The first international workshop of the European Leukemia Net (ELN) on standardization of FC in MDS was held in 2009 and updated in 2012. In 2014 it was extended to members from Australia, Canada, Japan, Taiwan and United States and international consensus recommendations (IMDSflow) were published [7–9], proposing minimal requirements for FC analysis of dysplasia and criteria for diagnostic and prognostic evaluation [7,9]. Bone marrow multiparametric FC gives complementary information for diagnosis and prognosis and it has been introduced as an important co-criteria for MDS diagnosis [4]. The World Health Organization (WHO) 2008 and 2016 recommendations highlight several FC aberrancies in maturation patterns as suggestive of MDS [21,22].

To the best of our knowledge this is the first published survey of current practice of FC for MDS diagnosis in Latin America, focusing on markers used and the information included in the report. We have also evaluated compliance with IMDSflow recommendations. Additionally, we have compared the results with those of Europe and USA/Canada. It is important to note that most European responses came from Spain and Netherlands, both countries with intense work production in the area of flow cytometry and MDS, which could be a potential source of bias of this study (Supplementary Table 1 s).

Centers from most countries in LA responded the survey. Surprisingly, the majority (60%) of FC laboratories in LA are well equipped with modern cytometers enabling the simultaneous analysis of 8 or more immunophenotypic markers, comparable to USA/Canada laboratories. Additionally, the number of samples received for MDS study in LA is as high as in USA/Canada and slightly lower than in Europe. The European laboratories that participated in the survey carry out a higher number of MDS samples per month, so they likely have more experience with FC in MDS diagnosis.

Although FC is expensive and not mandatory for MDS diagnosis, LA and European participants use a high number of markers (median 21) to study a suspected MDS case. However, compliance with IMDSflow recommendations [7–9] is poor and there is great heterogeneity in the markers used. Less than 10% of participants use the complete proposed panel. Moreover, around 50% of participants from LA and Europe use markers not suggested by IMDSflow but which are included in the EuroFlow panel (e.g CD35, CD36, CD64, CD300e, CD105). Therefore, these results may indicate that such laboratories are engaged in the EuroFlow standardization program and may explain the low compliance to IMDSflow recommendations. In addition, markers not included in the guidelines and with less evidence of diagnostic utility are frequently included, such as megakaryocytic markers that are used approximately 20% of participants [23].

Scoring systems have high sensitivity and acceptable specificity to discriminate MDS from other cytopenias [3]. Currently, many different FC scoring systems are available, with a wide variability in terms of the number of markers and parameters analyzed [9,24]. In this study, the use of any scoring system in the diagnostic work up of MDS was very low, despite the more frequent adoption by European participants.

The IMDSFlow suggest using the Ogata scoring system for screening purposes [8]. The Ogata score for low grade MDS was validated in a large multicenter study with good reproducibility, a sensitivity of 69% and specificity of 92% [25]. The current study shows that there is a wide variability in the use of this score by regions, ranging from no use in USA/Canada to around 25% in Europe (LA 10%).

It is recommended that the FC report should be part of an integrated diagnostic report, together with morphological, cytogenetic and/or molecular findings [7–9]. An interpretative comment should be added, stating whether the results are: consistent with MDS, shows limited number of changes seen in MDS or do not show MDS-related features [8,26]. Around 50% of the participants include in the report a general interpretation, however with differences among regions. Most participants include descriptive reports where they state the percentage of myeloid progenitor cells and aberrancies in the mature and immature compartments. Although a decrease in B-cell progenitor cells is frequently observed in MDS and is a useful parameter for diagnosis, only around 60% of the participants include the percentage of lymphoid progenitors in the report [12,27,28].

5. Conclusions

Taken together, the results of this survey highlight major heterogeneity in the application of FC to MDS diagnosis among the participants from LA, Europe and Canada/US, both in regards to the antibody combinations used and the information included in clinical diagnostic reports. Additionally, there is a low adherence to the IMDSflow recommendations and a scarce use of the scoring systems proposed that cannot be explained by economical restraints since the number of markers used is similar to those proposed in the guidelines and the majority of the centers owned modern cytometers. The next step will be the development of a FC consensus for MDS diagnosis in a cost-effective manner, suitable for the requirements for clinical practices in LA.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments

The authors thank the 265 participants who kindly respond to the survey. Without their cooperation this survey could not have been accomplished.

Additionally, the authors thank to the Grupo Latinoamericano de Mielodisplasia (GLAM), Grupo Rioplatense de Citometria de Flujo (GRCF), Brazilian Group of Flow Cytometry (GBCFLUX), Sociedad Peruana de Hematologia (SPH), International Clinical Cytometry Society (ICCS), European Society for Clinical Cell Analysis (ESCCA), International Society for Advancement of Cytometry (ISAC), Purdue University Cytometry mailing list, Dutch Cytometry Society and Iberian Cytometry Society (SIC) for their support and contribution to survey distribution.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.leukres.2019.01.009.

References

[1] L. Malcovati, S.D. Nimer, Myelodysplastic syndromes: diagnosis and staging, Cancer Control J. Moffitt Cancer Cent. 15 (Suppl) (2008) 4–13, https://doi.org/10.1177/107327480801504s02.

[2] P. Font, J. Loscertales, C. Soto, P. Ricard, C.M.- Novas, E. Martín-Clavero, M. López-Rubio, L. Garcia-Alonso, M. Callejas, A. Bermejo, C. Benavente, M. Ballesteros, T. Cedena, M. Calbacho, R. Urbina, J. Villarrubia, S. Gil, J.M. Bellón, J.L. Diez-Martin, A. Villegas, Interobserver variance in myelodysplastic syndromes with less than 5 % bone marrow blasts: unilineage vs. Multilineage dysplasia and reproducibility of the threshold of 2% blasts, Ann. Hematol. 94 (2015) 565–573, https://doi.org/10.1007/s00277-014-2252-4.

[3] C. Thiede, F. Buccisano, G.J. Schuurhuis, C.M. Aanei, T. Picot, E. Tavernier, D. Guyotat, L. Campos Catafal, Diagnostic utility of flow cytometry in myelodysplastic syndromes, Front. Oncol. 6 (2016), https://doi.org/10.3389/fonc.2016.00161 1613389–161.

[4] P. Valent, A. Orazi, D.P. Steensma, B.L. Ebert, D. Haase, L. Malcovati, A.A. Van De Loosdrecht, T. Haferlach, T.M. Westers, D.A. Wells, A. Giagounidis, M. Loken, A. Orfao, M. Lübbert, A. Ganser, W.-K. Hofmann, K. Ogata, J. Schanz, M.C. Béné, G. Hoermann, W.R. Sperr, K. Sotlar, P. Bettelheim, R. Stauder, M. Pfeilstöcker, H.-P. Horny, U. Germing, P. Greenberg, J.M. Bennett, Proposed minimal diagnostic criteria for myelodysplastic syndromes (MDS) and potential pre-MDS conditions Priority Review, Oncotarget 8 (2017) 73483–73500 www.impactjournals.com/oncotarget.

[5] D.A. Arber, A. Orazi, R. Hasserjian, J. Thiele, M.J. Borowitz, M.M. Le Beau, C.D. Bloomfield, M. Cazzola, J.W. Vardiman, The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia, Blood 127 (2016) 2391–2405, https://doi.org/10.1182/blood-2016-03-643544.

[6] P.L. Greenberg, R.M. Stone, A. Al-Kali, S.K. Barta, R. Bejar, J.M. Bennett, H. Carraway, C.M. De Castro, H.J. Deeg, A.E. DeZern, A.T. Fathi, O. Frankfurt, K. Gaensler, G. Garcia-Manero, E.A. Griffiths, D. Head, R. Horsfall, R.A. Johnson, M. Juckett, V.M. Klimek, R. Komrokji, L.A. Kujawski, L.J. Maness, M.R. O'Donnell, D.A. Pollyea, P.J. Shami, B.L. Stein, A.R. Walker, P. Westervelt, A. Zeidan, D.A. Shead, C. Smith, Myelodysplastic syndromes, version 2.2017, NCCN clinical practice guidelines in oncology, J. Compr. Canc. Netw. 15 (2017) 60–87, https://doi.org/10.6004/JNCCN.2017.0007.

[7] A.A. Van De Loosdrecht, C. Alhan, M.C. Béné, M.G. Della Porta, A.M. Dräger, J. Feuillard, P. Font, U. Germing, D. Haase, C.H. Homburg, R. Ireland, J.H. Jansen, W. Kern, L. Malcovati, J.G. Te Marvelde, G.J. Mufti, K. Ogata, A. Orfao, G.J. Ossenkoppele, A. Porwit, F.W. Preijers, S.J. Richards, G.J. Schuurhuis, D. Subirá, P. Valent, V.H.J. Van Der Velden, P. Vyas, A.H. Westra, T.M. De Witte, D.A. Wells, M.R. Loken, T.M. Westers, Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes, Haematologica 94 (8) (2009) 1124–1134, https://doi.org/10.3324/haematol.2009.005801.

[8] A. Porwit, A.A. Van De Loosdrecht, P. Bettelheim, L. Eidenschink Brodersen, K. Burbury, E. Cremers, M.G. Della Porta, R. Ireland, U. Johansson, S. Matarraz, K. Ogata, A. Orfao, F. Preijers, K. Psarra, D. Subirá, P. Valent, V.H.J. Van Der Velden, D. Wells, T.M. Westers, W. Kern, M.C. Béné, Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes - Proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS, Leukemia (2014) 1793–1798, https://doi.org/10.1038/leu.2014.191.

[9] T. Westers, R. Ireland, W. Kern, C. Alhan, J. Balleisen, P. Bettelheim, K. Burbury, M. Cullen, Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European LeukemiaNet Working Group, Leukemia. 26 (2012) 1730–1741, https://doi.org/10.1038/leu.2012.30.

[10] S.C. Reis-Alves, F. Traina, G. Harada, P.M. Campos, S.T.O. Saad, K. Metze, I. Lorand-Metze, Immunophenotyping in myelodysplastic syndromes can add prognostic information to well-established and new clinical scores, PLoS One 8 (2013) e81048, , https://doi.org/10.1371/journal.pone.0081048.

[11] A.A. Van De Loosdrecht, T.M. Westers, A.H. Westra, A.M. Drä Ger, V.H.J. Van Der Velden, G.J. Ossenkoppele, Identification of distinct prognostic subgroups in low- and intermediate-1–risk myelodysplastic syndromes by flow cytometry, Blood. 111 (2008) 1067–1077, https://doi.org/10.1182/blood-2007.

[12] A. Sternberg, S. Killick, T. Littlewood, C. Hatton, A. Peniket, T. Seidl, S. Soneji, J. Leach, D. Bowen, C. Chapman, G. Standen, E. Massey, L. Robinson, B. Vadher, R. Kaczmarski, R. Janmohammed, K. Clipsham, A. Carr, P. Vyas, Evidence for reduced B-cell progenitors in early (low-risk) myelodysplastic syndrome, Blood 106 (2005) 2982–2991, https://doi.org/10.1182/blood-2005-04-1543.

[13] K. Ogata, Y. Kishikawa, C. Satoh, H. Tamura, K. Dan, A. Hayashi, Diagnostic application of flow cytometric characteristics of CD34 + cells in low-grade myelodysplastic syndromes, Blood 108 (2006) 1037–1044, https://doi.org/10.1182/blood-2005-12-4916.

[14] R.W. McKenna, L.T. Washington, D.B. Aquino, L.J. Picker, S.H. Kroft, Immunophenotypic analysis of hematogones (B-lymphocyte precursors) in 662 consecutive bone marrow specimens by 4-color flow cytometry, Blood 98 (2001) 2498–2507, https://doi.org/10.1182/blood.V98.8.2498.

[15] J.J.M. van Dongen, L. Lhermitte, S. Böttcher, J. Almeida, V.H.J. van der Velden, J. Flores-Montero, A. Rawstron, V. Asnafi, Q. Lécrevisse, P. Lucio, E. Mejstrikova, T. Szczepański, T. Kalina, R. de Tute, M. Brüggemann, L. Sedek, M. Cullen, A.W. Langerak, A. Mendonça, E. Macintyre, M. Martin-Ayuso, O. Hrusak, M.B. Vidriales, A. Orfao, EuroFlow antibody panels for standardized n-dimensional flow cytometric immunophenotyping of normal, reactive and malignant leukocytes, Leukemia 26 (2012) 1908–1975, https://doi.org/10.1038/leu.2012.120.

[16] D.A. Wells, M. Benesch, M.R. Loken, C. Vallejo, D. Myerson, W.M. Leisenring, H.J. Deeg, Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation, Blood 102 (2003) 394–403, https://doi.org/10.1182/blood-2002-09-2768.

[17] S. Mathis, N. Chapuis, C. Debord, A. Rouquette, I. Radford-Weiss, S. Park, F. Dreyfus, C. Lacombe, M.B. Né, O. Kosmider, M. Fontenay, V. Bardet, Flow cytometric detection of dyserythropoiesis: a sensitive and powerful diagnostic tool for myelodysplastic syndromes, Leukemia 27 (2013) 1981–1987, https://doi.org/10.1038/leu.2013.178.

[18] M.G. Della Porta, E. Travaglino, E. Boveri, M. Ponzoni, L. Malcovati, E. Papaemmanuil, G.M. Rigolin, C. Pascutto, G. Croci, U. Gianelli, R. Milani, I. Ambaglio, C. Elena, M. Ubezio, M.C. Da Via', E. Bono, D. Pietra, F. Quaglia, R. Bastia, V. Ferretti, A. Cuneo, E. Morra, P.J. Campbell, A. Orazi, R. Invernizzi, M. Cazzola, Rete Ematologica Lombarda (REL) Clinical Network, Minimal morphological criteria for defining bone marrow dysplasia: a basis for clinical implementation of WHO classification of myelodysplastic syndromes, Leukemia 29 (2015) 66–75, https://doi.org/10.1038/leu.2014.161.

[19] U. Bacher, E. Shumilov, J. Flach, N. Porret, R. Joncourt, G. Wiedemann, M. Fiedler, U. Novak, U. Amstutz, T. Pabst, Challenges in the introduction of next-generation sequencing (NGS) for diagnostics of myeloid malignancies into clinical routine use, Blood Cancer J. 8 (2018) 113, https://doi.org/10.1038/s41408-018-0148-6.

[20] L. Malcovati, M.G. Della Porta, C. Pascutto, R. Invernizzi, M. Boni, E. Travaglino, F. Passamonti, L. Arcaini, M. Maffioli, P. Bernasconi, M. Lazzarino, M. Cazzola, Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making, J. Clin. Oncol. 23 (2005) 7594–7603, https://doi.org/10.1200/JCO.2005.01.7038.

[21] S.H. Swerdlow, E. Campo, N.L. Harris, E.S. Jaffe, S.A. Pileri, H. Stein, J. Thiele, J.W. Vardiman, WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, (2008).

[22] J.M. Bennett, Changes in the updated 2016: WHO classification of the myelodysplastic syndromes and related myeloid neoplasms, Clin. Lymphoma Myeloma Leuk. 16 (2016) 607–609, https://doi.org/10.1016/j.clml.2016.08.005.

[23] A.F. Sandes, M. Yamamoto, S. Matarraz, Md.L.L.F. Chauffaille, S. Quijano, A. Lopez, T. Oguro, E.Y.S. Kimura, A. Orfao, Altered immunophenotypic features of peripheral blood platelets in myelodysplastic syndromes, Haematologica 97 (2012) 895–902, https://doi.org/10.3324/haematol.2011.057158.

[24] L.C. Bento, R.P. Correia, C.L. Pitangueiras Mangueira, R. De Souza Barroso, F.A. Rocha, N.S. Bacal, L.C. Marti, The use of flow cytometry in myelodysplastic syndromes: a review, Front. Oncol. 7 (2017) 270, https://doi.org/10.3389/fonc.2017.00270.

[25] M.G. Della Porta, C. Picone, C. Pascutto, L. Malcovati, H. Tamura, H. Handa, M. Czader, S. Freeman, P. Vyas, A. Porwit, L. Saft, T.M. Westers, C. Alhan, C. Cali, A.A. van de Loosdrecht, K. Ogata, Multicenter validation of a reproducible flow cytometric score for the diagnosis of low-grade myelodysplastic syndromes: results of a European LeukemiaNET study, Haematologica 97 (2012) 1209–1217, https://doi.org/10.3324/haematol.2011.048421.

[26] A.A. van de Loosdrecht, T.M. Westers, Cutting edge: flow cytometry in myelodysplastic syndromes, J. Natl. Compr. Canc. Netw. 11 (2013) 892–902, https://doi.org/10.6004/jnccn.2013.0106.

[27] K. Ogata, Y. Kishikawa, C. Satoh, H. Tamura, K. Dan, A. Hayashi, Diagnostic application of flow cytometric characteristics of CD34+ cells in low-grade myelodysplastic syndromes, Blood 108 (2006) 1037–1044, https://doi.org/10.1182/blood-2005-12-4916.

[28] S. Maftoun-Banankhah, A. Maleki, N.J. Karandikar, A.A. Arbini, F.S. Fuda, H.-Y. Wang, W. Chen, Multiparameter flow cytometric analysis reveals low percentage of bone marrow hematogones in myelodysplastic syndromes, Am. J. Clin. Pathol. 129 (2008) 300–308, https://doi.org/10.1309/4W2G3NDXUPG5J33N.