Sensitivity of Blood Cells' Maturation Pattern in the Diagnosis of Myelodysplastic Syndrome Using Flow Cytometry - a Systematic Review

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Abstract

Clinical history, morphological appearance and cytogenetic data are required in identifying cases of Myelodysplastic Syndrome (MDS). However, this clonal stem cell disorder is still widely heterogenous and multiple tools are utilized in determining the diagnosis and prognosis. The current approaches in diagnosis are inherently subjective and lack of sensitivity. Over the years, altered maturation patterns using flow cytometry analysis have been reported to be useful for identification of MDS. This systematic review aims to assess the sensitivity of maturation pattern in obtaining MDS diagnosis. Electronic databases (MEDLINE, PROQUEST, OVID, Scopus, Web of Science) searched were yielded 677 articles. Snowballing was also employed. Two reviewers assessed each article independently using the following inclusion criteria: all types of MDS; WHO or FAB classification; diagnosis; immunophenotyping. Nineteen papers that met our inclusion criteria were analysed on the maturation pattern using flow cytometry. Samples used were bone marrow aspiration and prepared using either whole blood lysis or Ficoll-density gradient centrifugation. The most studied lineage in diagnosing MDS using maturation pattern is myeloid mainly looking on the CD34+, CD11b/CD16, CD13/CD16 and CD235a/CD71 expression pattern. Five studies showed sensitivity between 70 to 98 percent. Maturation pattern has shown high sensitivity and may be used as an ancillary technique for the diagnosis of MDS. However, flow cytometry strategies employed lack of standardization in assay, scoring system, and on how flow cytometry data have been analysed. Thus, more study need to be done within laboratory and multi-centre study to ensure validity and reliability of maturation pattern as an adjunct test.

Keywords Flow cytometry, normal, bone marrow, maturation

1. Introduction

Myelodysplastic syndrome (MDS) is a heterogenous group of clonal bone marrow disorders in which the bone marrow produce abnormal blood cells. The hallmark features of MDS are manifestation of peripheral cytopenia and dysplasia which may range from asymptomatic to anaemia, recurrent infection or easy bruising (Aster & Stone, 2015). Thirty percent of MDS patients have the risk of developing acute myeloid leukemia (AML) (Bejar & Steensma, 2014; Mufti et al., 2008)

The current gold standard method for the diagnosis of MDS is by morphological evaluation which is complemented by clinical history, and cytogenetic abnormalities (Porwit et al., 2014; Westers et al., 2012). However, morphological evaluation may be very subjective and is highly dependent on the quality of peripheral blood and bone marrow smear sampling. Moreover, morphologic dysplasia is not specific for MDS and can also be seen in a wide range of non-MDS cytopenic conditions. Other than that, cytogenetic abnormalities are infrequent in low grade MDS (Stachurski et al., 2008; M Stetler-Stevenson & Yuan, 2009). In cases with absence of significant marrow dysplasia and normal cytogenetics, it may be difficult to ensure the diagnosis of MDS (DeZern & Sekeres, 2014; Kern, Haferlach, Schnittger, Alpermann, & Haferlach, 2013;

Killick et al., 2014). Therefore, there is a need for other methods which are more objective to make the diagnosis.

In the past 20 years, flow cytometry immunophenotyping remains an important tool for the diagnosis of hematologic malignancies (Kalina et al., 2012). The advancement in flow cytometry instrumentation and broadening range of antibodies and fluorochromes available have improved identification of abnormal population clonal disorder patients (Craig & Foon, 2008). Qualitative approach in flow cytometry using pattern recognition in addition to quantitative approach may enhanced diagnostic and prognostic abilities. The pattern-recognition is based on the assessment of a deviation from the normal antigen expression pattern (Chopra et al., 2012; Kussick et al., 2005). Abnormalities may be identified by comparing patterns of normal cell with the patterns of antigen expression on pathologic cell population. Maturation and differentiation of hematopoietic cells is tightly controlled in which antigen expression pattern are highly conserved (Reis-Alves, Traina, Metze, & Lorand-Metze, 2015). Therefore, application of pattern recognition to diagnose MDS may prove to be indispensable.

Since the past 15 years, a number of studies have applied the maturation pattern to the study and diagnosis of MDS. The ability to identify the abnormal expression of antigens have been repeatedly demonstrated (Kern, et al, 2010; Westers et al., 2012). This paper aims to systematically review the sensitivity of maturation pattern used in diagnosing MDS. Sensitivity of maturation pattern was assessed based on the strategies employed in sample preparation, cell population studied, antibodies used and analytic approaches employed.

2. Methodology

Search strategy

Electronic searches were performed from Medline, Proquest, Ovid, Scopus and Web of Science with unlimited publication date. The keywords used were ("Myelopdysplastic Syndrome" OR "MDS" OR "Myelodysplas*") AND (cell maturation) AND (maturation pattern). Additional records were also identified by snowballing. No limits were applied for language.

Eligibility criteria

This review includes all experimental studies that had been published from 1967 until 2015 without restriction by any publication status or language. Editorial papers, reviews, reports and books were excluded. The inclusion criteria were: 1) studies that evaluated MDS patients based on maturation pattern, 2) all subtypes of MDS either FAB or WHO classification, 3) immunophenotyping evaluation on MDS sample. The exclusion criteria were: 1) study on normal bone marrow, 2) other clonal disorders, 3) transplantation or regeneration, and 4) patient on treatment or therapy.

Study selection

All articles that met the inclusion criteria were compiled using the software EndNote (VersionX7.0.1). After the removal of duplicates, two authors independently screened the titles and abstracts according to the inclusion and exclusion criteria. When it was not clear whether the abstract met the inclusion criteria, the full article was reviewed. Disagreements between reviewers were resolved by consensus.

Data extraction

Data were extracted into a specifically designed spreadsheet and included details on the number and types of sample, flow cytometry setting, studied cell lineage, sample preparation, antigen markers and outcome of the study. Report of the study was done according to PRISMA guidelines.

3. Results

Study selection

The search strategies yielded 753 citations from the five databases. An additional 11 citations were identified by snowballing. After duplicates were removed, 688 citations remained. Of these, 663 studies were discarded after reviewing the title and abstract. The full text of the remaining 25 citations was examined. Six studies did not meet the inclusion criteria and only 19 studies were finally selected for this systematic review.

Study characteristics

- Study setting

Out of nineteen studies, seven studies were conducted in America, followed by Europe (7), Southeast Asia (2), South America (1), East Asia (1) and South Asia (1). These studies were carried out prospectively or retrospectively and all the 19 studies were single-centred studies.

Samples

All studies used bone marrow aspirates (BMA) as their sample. Six articles used samples from suspected MDS and thirteen studies used BMA from definite MDS sample. There were also studies that include non-diagnostic MDS (NDM), CML and CMML sample. NDM sample is defined as a sample that lacked dyspoiesis and where aspirates smears were not available.

- MDS classification

All samples were classified based on WHO classification, except studies by Stetler-Stevenson et al. (2001), Broojerdi, Ramasamy, Noor, & Seman (2012), Lorand-Metze, Ribeiro, Lima, Batista, & Metze.(2007) and Malcovati et al. (2005) who used the French-American-British (FAB) classification at first analysis and then reclassified based on WHO classification. Only Kern, et al. (2010) used both FAB and WHO to subgroup MDS. While Pirucello, Young & Aoun., (2006), Monaghan, Surti, Doty, & Craig (2012), Chopra et al. (2012) and Hanumanthu & Pirucello (2013) did not mention the MDS classification used.

Methodology

Flow cytometry analysis

Maturation pattern of bone marrow aspiration from MDS patients were evaluated using flow cytometry for all selected articles. One study used Navios flow cytometer (Beckman Coulter) for analysis using six-color staining (Mathis et al., 2013). Coulter FC500 was used for analysis using five-color staining (Chopra et al., 2012; Hanumanthu & Pirruccello, 2013; Kern et al., 2010; Pirruccello, t al., 2006) and four-color staining (Stachurski et al., 2008). Coulter EPICS XL (De Smet

et al., 2012; Della Porta et al., 2006) and EPICS XL-MCL (Vikentiou et al., 2009) flow cytometer were used to analyse four-color staining. Four-color staining was also analysed using FACSCalibur in a number of studies (Maftoun-Banankhah et al., 2008; Malcovati et al., 2005; Matarraz et al., 2008; Monaghan, et al, 2012) and FACSCanto (Broojerdi, et al., 2012; Chung et al., 2012). FACSCan flow cytometer was used to analyse three-color staining (Maftoun-Banankhah et al., 2008; Stetler-Stevenson et al., 2001).

Sample preparation

For immunophenotyping analysis, the patients' sample were prepared either by lysing the red cell or using density centrifugation. Three studies used Ficoll density centrifugation to separate the mononuclear cells without lysis. In contrast, twelve studies lysed the mature red cells to minimize loss of selective mononuclear cell such as granulocytes. However, a study by Kern et al. (2010) used centrifugation method and then was followed by red cell lysis. While, another three studies did not mentioned their sample preparation method.

Type of cell lineage and antigen markers analyzed

The antigen markers used in the studies were based on the type of cell population. Ten (52.6%) articles studied on various cell lineages in the bone marrow comprising of granulocytic, monocytic, erythroid and lymphoid lineages including the blast cell population. Among these ten studies only two studies examined the lymphoid cell. Della Porta et al (2006) and Mathis et al. (2013) specified their observations on the erythroid lineage. Pirucello et al. (2006), Matarraz et al. (2008) and De Smet et al. (2012) studied on the blast population. While Vikentiou et al. (2009) and Monaghan et al (2012) were focused on the granulocytic lineage. From this review, only Maftoun-Banankhah et al. (2008) looked at the hematogones. Hanumanthu & Pirucello (2013) looked at the expression of granulocyte colony-stimulating factor receptor (CD114) in MDS.

In thirteen articles that studied the blast population, seven (53.8%) papers used CD34 as antigen markers. The remaining six (46.2%) papers did not mention how they defined blast population. CD117 and HLA-DR also were used in eight (61.5%) and five (38.5%) of these papers as additional markers to identify precursor cells, respectively. In myeloid maturation, CD13, CD11b, CD16, CD33, HLA-DR and CD10 were used in twelve (92.3%), eleven (84.6%), ten (76.9%), nine (69.2%), seven (53.8%), and five (38.5%) papers out of thirteen articles, respectively. In nine articles that studied on erythroid lineage, nine (100%) used CD71 and seven (77.8%) papers used CD235a or glyphorin A (GlyA) as antigen markers. Only one (11.1%) paper looked at CD105, H ferritin (HF), L ferritin (LF) and mitochondrial ferritin (MtF) expression in dyserythropoiesis of MDS cases.

Quality assessment

A full description of MDS definition using flow cytometry and cut off point are essential information in assessment on the validity of the test as it may influence the interpretation of test results. In total, six (31.6%) articles used suspected MDS sample. Three (50%) of these papers, described how they define MDS using flow cytometry, whereas one (16.6%) paper described without citation. In total, sixteen (84.2%) papers measured quantitative data in diagnosing MDS and only four (25%) papers described diagnostic cut off points. The remaining twelve (75%)

articles did not mention the cut off points and only compared the mean (or median) of control and MDS sample.

Antigen expression pattern

Antigen expression pattern were evaluated based on blast, granulocytic, monocytic and erythroid lineage. The common abnormalities in MDS demonstrated by all the nineteen studies are shown in Table 1. Study on myeloid maturation demonstrated abnormal pattern of CD11b/CD16 and CD13/CD16 in five papers. Decreased or asynchronous expression of CD16 and CD11b was shown in five (50%) out of ten papers and five (45.5%) out of eleven papers, respectively. Abnormal or increased expression of CD13 was shown in three (25%) out of twelve papers (Chopra et al., 2012; Kussick et al., 2005; Lorand-Metze, Ribeiro, Lima, Batista, & Metze, 2006). CD33 showed decreased or negative expression in four (33.3%) out of nine papers (Broojerdi et al., 2012; Kern et al., 2010; Kussick et al., 2005; Stachurski et al., 2008). Four (80%) out of five papers showed decreased of CD10 in mature granulocytes (Chung et al., 2012; Lorand-Metze, et al., 2006; Malcovati et al., 2005; Stachurski et al., 2008). Eight studies also showed overexpression of CD56 in monocyte. Decreased or low side scatter (SSC) or hypogranulation was demonstrated in six (50%) out of twelve papers (Broojerdi et al., 2012; Chopra et al., 2012; Kussick et al., 2005; Lorand-Metze et al., 2006; Stachurski et al., 2008; Stetler-Stevenson et al., 2001). In twelve articles that study on blast population, nine (75%) studies showed high percentage of blast cells in MDS compared to normal control (Chopra et al., 2012; De Smet et al., 2012; Kern et al., 2010; Malcovati et al., 2005; Matarraz et al., 2008; Monaghan et al., 2012; Pirruccello et al., 2006; Stachurski et al., 2008). Nine (100%) papers demonstrated decreased expression of CD71 and CD235a in MDS cases (Boroojerdi et al., 2013; Broojerdi et al., 2012; Della Porta et al., 2006; Kern et al., 2010; Lorand-Metze et al., 2006; Malcovati et al., 2005; Stetler-Stevenson et al., 2001) . Numerical assessment based on CD71 MFI in two (22.2%) studies also showed a significant reduction compared to the control group (Chopra et al., 2012; Mathis et al., 2013). Five (100%) studies also showed aberrant expression of lymphoid antigens such as, CD2, CD5, CD7 or CD56 on blasts population (Chopra et al., 2012; Kussick et al., 2005; Pirruccello et al., 2006; Stachurski et al., 2008; Stetler-Stevenson et al., 2001).

Table 1 Common abnormalities in MDS

Type of lineage	Abnormalities in MDS	Authors
Blast	High percentage (>3%)	Malcovati et al., 2005; Pirucello et al., 2006; Stachurski et al., 2008; Matarraz et al., 2008; Kern et al., 2010; Chopra et al., 2012; Monaghan et al., 2012;De Smet et al., 2012
	Presence of lineage infidelity markers	Stetler-Stevenson et al., 2001; Kussick et al., 2005; Pirucello et al., 2006; Stachurski et al., 2008; Chopra et al., 2012
Granulocyte	Decreased SSC	Stetler-Stevenson et al., 2001; Kussick et al., 2005; Lorand-Metze et al., 2006; Stachurski et al. 2008; Chopra et al., 2012; Broojerdi et al., 2012
	Asynchronous CD13/CD16/CD11b pattern	Stetler-Stevenson et al. 2001; Malcovati et al., 2005; Stachurski et al., 2008; Kern et a., 2010; Chung et al., 2012
	Decreased expression of CD16, CD11b, CD33, CD10	Kussick et al. 2005; Lorand-Metze et al., 2006; Vikentiou et al., 2009; Broojerdi et al. 2012; Chung et al., 2012; Broojerdi et al., 2013

	Asynchronous or increased CD13 expression	Kussick et al., 2005; Lorand-Metze et al., 2006; Chopra et al.,2012
	Aberrant CD56 expression	Kussick et al., 2005; Pirucello et al., 2006
Monocyte	Aberrant expression of HLA-DR, CD11b, CD13, CD14, CD33, CD64	Stetler-Stevenson et al., 2001; Kussick et al., 2005; Lorand-Metze et al., 2006; Stachurski et al., 2008; Kern et al., 2010; Chopra et al., 2012; Broojerdi et al., 2012; Broojerdi et al., 2013
	Overexpression of CD56	Stetler-Stevenson et al., 2001; Kussick et al., 2005; Lorand-Metze et al., 2006; Stachurski et al., 2008; Kern et al., 2010; Chopra et al., 2012; Broojerdi et al., 2012; Broojerdi et al., 2013
Erythroid cell	Decreased CD71 and CD235a expression	Stetler-Stevenson et al., 2001; Malcovati et al., 2005; Della Porta et al., 2006; Lorand-Metze et al., 2006; Kern et al., 2010; Broojerdi et al., 2012; Broojerdi et al., 2013
	Low CD71 MFI	Chopra et al, 2012; Mathis et al., 2013

Sensitivity of maturation pattern in MDS

Out of nineteen studies, only five studies evaluated the sensitivity of maturation pattern for diagnosing MDS. Sensitivity of maturation pattern was determined by comparing them with cytomorphology and cytogenetics evaluation. Kussick et al. (2005) used more than 15 parameters and showed overall sensitivity of 95% and specificity of 67%. Another study by Della Porta et al. (2006) used six parameters on erythroid lineage which showed 97.2% sensitivity and 100% specificity. They also looked at the MDS classification with 100% sensitivity and 94.7% specificity for patients with ring sideroblast. Stachurski et al. (2012) define MDS into three categories: negative, intermediate, and positive. Intermediate MDS is defined as abnormal level of CD117 and CD45 in blast, presence or absence of myelomonocytic antigen alterations or absence of abnormality at blast but with at least three mature myelomonocytic antigen alterations. Inclusion of the intermediate category as indication of MDS by Stachurski et al. (2008) showed increased in sensitivity from 84% to 98% but decreased specificity from 97% to 78%. Another study by Chopra et al. (2012) showed sensitivity and specificity based on scoring by Stachurski et al. (2008) as 92.59% and 96.67%, respectively. While Mathis et al. (2013) have diagnosed MDS using RED-score with sensitivity of 77.5% and specificity of 90%. RED-score was developed based on CD71 MFI, CD36 MFI and haemoglobin level. Ogata MFC-score was designed with aimed to be reproducible in numerous laboratories and for low-grade MDS identification. With combination of RED-score and Ogata MFC-score, the sensitivity was significantly increased (87.9%), whereas the specificity remained high (88.9%).

4. Conclusion

In conclusion, maturation pattern can be useful to distinguish MDS with non-clonal disorders and could be helpful to distinct MDS among subgroup. This approach able to identify dual or multiple abnormal antigen expression in MDS which demonstrated high sensitivity of more than 80% and specificity more than 67%. However, flow cytometry strategies employed lack of standardization in assay, scoring system, and on how flow cytometry data have been analysed. Thus, more study need to be done within laboratory and multi-centre study to ensure validity and reliability of maturation pattern as an adjunct test.

Second, the findings shows the hedging in foreign purchases, although relatively less in value as compared to hedging in foreign sales, has a positive effect on firm equity returns when exchange rate of foreign purchase become more volatile but this is not for hedging in foreign sales. These manufacturing companies actually enjoy reducing cost of imports in raw material or collect handsome proceed from their derivative contracts. This positive outcome shows the evidence how manufacturing companies can achieve higher equity return as investor might build confidence in companies that practice effective treasury management.

Third, we show that multiple foreign currency exposure either in foreign sales or foreign purchases do not have any effect on equity return. The manufacturing company might have structure its operations so that they can operationally hedged against the foreign currency exposure by diversifying the import operations with export sales. This study however offer no further precise picture of how Malaysian manufacturing companies actually performs these natural hedging and thus further study is warranted to draw a conclusion on this.

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