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Standardized fluorescence in situ hybridization testing based on an appropriate panel of probes more effectively identifies common cytogenetic abnormalities in myelodysplastic syndromes than conventional cytogenetic analysis: A multicenter prospective study of 2302 patients in China

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ABSTRACT

In an attempt to establish the advantages of fluorescence in situ hybridization (FISH) studies over conventional cytogenetic (CC) analysis, a total of 2302 de novo MDS patients from 31 Chinese institutions were prospectively selected in the present study for both CC and standardized FISH analysis for +8, -7/7q-, -5/5q-, 20q- and–Y chromosomal abnormalities. CC analysis was successful in 94.0% of the patients; of these patients, 35.9% of the cases were abnormal. FISH analysis was successful in all 2302 patients and detected at least one type of common cytogenetic abnormality in 42.7% of the cases. The incidences of +8, -7/7q-, -5/5q-, 20q- and–Y chromosomal abnormalities by FISH were 4.1% to 8.7% higher than those by CC. FISH identified abnormalities in 23.6% of the patients exhibiting normal CC results and revealed that 20.7% of the patients with adequate normal metaphases (≥20) had abnormal clones. FISH identified cytogenetic abnormalities in 50.4% of the patients with failed CC analysis. In summary, our multicenter studies emphasised and confirmed the importance of applying standardized FISH testing based on an appropriate panel of probes to detect common cytogenetic abnormalities in Chinese de novo MDS patients, particularly those with normal or failed CC results.

1. Introduction

Cytogenetic information is essential to the diagnosis, classification, and prognostic assessment of de novo myelodysplastic syndromes (MDS). Traditionally, this information could be obtained only by conventional cytogenetic (CC) analysis. Cytogenetic abnormalities are detected in 35–60% of de novo MDS patients by CC analysis, and the most frequent chromosomal abnormalities are the deletion of 5q (5q−), trisomy 8 (+8), the deletion of 20q (20q−), monosomy of chromosome 7 (−7), the deletion of 7q (7q−), monosomy of chromosome 5 (−5) and the loss of chromosome Y (−Y) [1–3]. Although CC analysis has been reliably used for karyotyping and is the gold standard for detecting chromosomal abnormalities, it has some limitations. CC analysis requires dividing cells from the neoplastic clone, and it lacks sensitivity. Moreover, CC analysis alone might not be informative if insufficient numbers of metaphase cells are obtained for examination because a specimen is hypocellular or expands poorly in culture.

Fluorescence in situ hybridization (FISH), which does not require dividing cells and can be easily quantified, is increasingly being used to identify specific cytogenetic aberrations in MDS [4–18]. The primary advantage of FISH is its greater sensitivity than CC analysis, resulting from the analysis of a greater number of interphase cells. However, the clinical role of FISH studies and the context in which FISH might provide additional information that is undetectable by CC analysis remain uncertain. Prior studies comparing CC and FISH panel testing in MDS have yielded conflicting results [4–18]. Several studies have demonstrated that the combination of FISH and CC analysis significantly improved the detection of abnormalities in the cytogenetic diagnosis of MDS [4–11]. Conversely, other studies have reported that FISH detected 6% or fewer additional abnormalities compared to CC analysis alone, and these studies suggested limiting FISH testing to cases with suboptimal karyotyping [12–18]. The examination of small sample sizes and the use of data collected from a single center might have contributed to these differing results. Therefore, the analysis of a large cohort of multicenter MDS patients is needed to determine whether FISH analysis has additional value in the evaluation of MDS.

In an attempt to establish the advantages of FISH over CC analysis, we prospectively compared metaphase karyotyping and a standardized panel of FISH analysis in 2302 bone marrow specimens from de novo MDS patients from 31 Chinese hospitals. We found that standardized FISH testing was more effective in identifying common cytogenetic abnormalities than CC analysis and that FISH testing added information to the cytogenetic evaluation in patients with normal or failed CC analysis, even in those with sufficient normal metaphases.

2. Materials and methods

2.1. Patients

Between January 2011 and March 2013, 2302 newly diagnosed de novo MDS patients from 31 hospitals in China were enrolled in the study. All of the patients met the minimal diagnostic criteria for MDS, according to a consensus statement from a working conference [19]. All of the patients were classified according to the French–American–British classification (FAB) [20] as well as the World Health Organization (WHO 2008) classification. The clinical characteristics of the patients are shown in Table 1. The bone marrow samples for CC analysis and FISH testing were obtained at diagnosis in all of the patients. This study was approved by the local ethics review committees of our institutions. This trial was registered at www.chictr.org as ChiCTR-ONRC-11001709. All of the subjects provided written informed consent.

2.2. Conventional cytogenetic analysis

Bone marrow metaphase cytogenetic studies were performed on 24-h bone marrow (BM) cultures with or without the addition of granulocyte colony-stimu1ating factor. The cells were cultured in RPMI 1640 supplemented with 20% foetal calf serum and 2% L-glutamine. The cells were harvested, and cell suspensions were stored in a freezer at approximately −20 °C before conventional cytogenetic karyotyping and FISH studies were performed. Conventional cytogenetic karyotyping was performed using standard G-banding or R-banding cytogenetic methods. Whenever possible, 20 metaphases were analysed. The karyotypes were described according to ISCN 2009 [21].

Table 1
Clinical parameters at presentation in 2302 MDS patients.

Variable	No. (%) of patients
Patient age, *y*, range (median)	6–92 (52)
Sex: male/female	1345 (58.4)/957(41.6)
FAB classification	
RA	1441 (62.6)
RARS	129 (5.6)
RAEB	631 (27.4)
CMML	57 (2.5)
RAEBT	44 (1.9)
WHO classification	
RCUD	713 (31.0)
RARS	112 (4.9)
RCMD	636 (27.6)
$RAEB_1$	357 (15.5)
$RAEB_2$	274 (11.9)
5q− syndromes	26 (1.1)
MDS-MPN	59 (2.6)
MDS-U	81 (3.5)
AML	44 (1.9)

RA: refractory anemia; RARS: refractory anemia with ring sideroblasts; RAEB: refractory anemia with excess blasts; CMML: chronic myelomonocytic leukemia; RAEBT: RAEB in transformation; RCUD: refractory cytopenia with unilineage dysplasia; RCMD: refractory cytopenia with multilineage dysplasia; MPN: myeloproliferative neoplasm; MDS-u: MDS-unclassifiable; AML: acute myeloid leukemia.