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HAEMATOLOGY

Prognostic evaluation of ALIP and CD34 immunostaining in IPSS-R subgroups of myelodysplastic syndromes

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Summary

In order to evaluate the prognostic value of abnormal localisation of immature precursors (ALIP) and CD34 immunostaining in myelodysplastic syndromes (MDS), bone marrow histopathological features in 187 MDS patients were retrospectively analysed and the prognostic significance of ALIP and CD34 immunostaining on overall survival (OS) and progression to leukaemia-free survival (PFS) in total patients and different Revised-International Prognostic Scoring System (IPSS-R) subgroups were evaluated. In univariate analysis, age ≥60, ALIP, ≥5% CD34+ cells, CD34+ clusters and IPSS-R subgroups were associated with shorter OS ($p = 0.027$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, respectively) and PFS ($p = 0.029$, $p = 0.006$, $p = 0.001$, $p < 0.0001$, $p < 0.0001$, respectively). Haemoglobin level had a significant impact on OS ($p < 0.0001$) but not on PFS ($p = 0.054$). In multivariate analysis, ALIP, haemoglobin level, ≥5% CD34+ cells, CD34+ clusters and IPSS-R subgroups had independent influence on OS ($p = 0.012$, $p < 0.0001$, $p = 0.010$, $p < 0.0001$, $p < 0.0001$, respectively), while only CD34+ clusters and IPSS-R subgroups had independent influence on PFS ($p < 0.0001$, $p = 0.016$, respectively). In different IPSS-R subgroups, ALIP could maintain its prognostic impact in lower IPSS-R risk subgroups, while ≥5% CD34+ cells and CD34+ clusters had significant prognostic value in both lower and intermediate-higher IPSS-R risk subgroups. Therefore, CD34+ clusters showed more important prognostic impact on survival and progression to leukaemia.

Key words: Bone marrow biopsy; myelodysplastic syndromes; Revised-International Prognostic Scoring System; prognosis; overall survival; leukaemia-free survival.

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INTRODUCTION

Myelodysplastic syndromes (MDS) are a group of clonal disorders characterised by cytopenia and dysplasia in haematopoietic lineages caused by ineffective haematopoiesis with risk of progression to acute leukaemia (AL) or bone marrow failure.[1–3] Accurate assessment of the prognosis in individual patients is required to make proper treatment decisions due to high variability in clinical outcome in MDS patients.[4–6]

Bone marrow biopsy supplemented by immunohistochemistry has shown its important diagnostic value in MDS,[7,8] and the prognostic value of some parameters of bone marrow histopathology such as bone marrow fibrosis, abnormal localisation of immature precursors (ALIP) and CD34+ clusters in MDS have been investigated.[9–12] However, there has been a lack of understanding of the prognostic role of histopathology in subtypes of different Revised-International Prognostic Scoring System (IPSS-R) risk of MDS. Therefore, we analysed the bone marrow histomorphological features in 187 MDS patients, investigated their relations with World Health Organization (WHO) classification, and mainly investigated the prognostic significance of ALIP and CD34 immunostaining in IPSS-R subgroups.

PATIENTS AND METHODS

Patients

Between January 2007 and December 2013, 187 consecutive MDS patients at the Haematology Department of Zhongnan Hospital of Wuhan University and Wuhan Union Hospital, China, were analysed. Routine blood tests, peripheral blood smears, bone marrow aspirates, bone marrow biopsies and chromosome examinations were required for diagnosis. The diagnosis and classification of MDS cases were initially classified according to the WHO 2002 criteria.[2] This observational study was approved by the institutional ethics committee.

Bone marrow biopsy

Bone marrow core biopsy specimens (0.3 × 1.5 cm) were obtained by biopsy needle from posterior superior iliac spine, fixed in 10% formalin, dehydrated by ethanol, and decalcified by concentrated nitric acid. Paraffin embedding followed and, after cutting into slices, the bone marrow biopsy section was stained by hematein-Giemsa-fuchsine (HGF) and Gomori argentation was performed for evidence of fibrosis. CD34 immunostaining was performed as previously described.[13] Paraffin embedded sections were immunostained for CD34 antigen, using the QBEND10 monoclonal antibody (Cat 1185; Immunotech, France). CD34 immunostaining in cell surface membrane and cytoplasm were counted.

Each biopsy was evaluated for the following four aspects:

1. Cellularity was assessed according to European consensus guidelines,[14] including hypercellularity (haematopoietic

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cells occupying more than 50% of intertrabecular spaces), normocellularity (30–50%) and hypocellularity (<30%).
2. The proliferation degree of fibrosis was assessed according to European consensus guidelines,[14] including grade 0 (absence of fibrosis), grade 1 (mild fibrosis), grade 2 (moderate), and grade 3 (severe).
3. Dysplasia included ALIP (defined as the aggregates of three or more myeloblasts or promyelocytes clusters distributed through the intertrabecular area), hot spot (defined as the aggregation of more than 20 erythroblasts at the same phase), mononuclear megakaryocytes and micromegakaryocytes. ALIP positive was defined as at least three aggregates located away from the endosteal surface.[9]
4. The percentage of CD34+ cells in all nucleated cells was assessed by counting in 20 or more fields at 1000 magnification. CD34+ cluster was defined as aggregates of three or more positive cells. A case with at least three CD34+ cell clusters was considered positive for this feature.[11]

Statistical analysis

The follow-up interval was from diagnosis to death or the last follow-up date (i.e., 10 December 2014). The overall survival (OS) was measured from the time of diagnosis until the date of death or until the last follow-up date. The progression to leukaemia-free survival (PFS) was measured from the time of diagnosis to the date of leukaemia transformation or the last day of follow up (censored data). The Kaplan–Meier method was used to evaluate the probability of survival. The log-rank test was applied to compare survival and cumulative risk of progression to AL between subgroups of each factor. The Cox proportional hazard regression model was applied for univariate and multivariate analysis assessing OS and PFS. The multivariable Cox model with stepwise selection was used to confirm independent prognostic factors. Hazard ratios (HR) and their 95% confidence intervals (CI) were calculated. A p value less than 0.05 was considered statistically significant. All statistical analysis was carried out using SPSS 15.0 software (SPSS, USA).

RESULTS

Patient characteristics

Tables 1 and 2 present the clinical and bone marrow pathological characteristics of the total 187 patients. The median age was 51.9 years (range 9–88 years), 47 patients (25.0%) were over 60 years of age, and 108 patients (57.8%) were male. Biopsy showed normocellularity in 56 cases (29.9%), hypercellularity in 104 cases (55.6%), and hypocellularity in 27 cases (14.4%). Hypercellularity was found more frequently in refractory anaemia with excess blasts (RAEB) patients (46/64, 71.9%) than in other subgroups in our series, and there was a statistical difference when compared with other subtypes [$p = 0.027$ versus refractory anaemia (RA) and refractory anaemia with ringed sideroblasts (RARS); $p = 0.009$ versus refractory cytopenia with multilineage dysplasia (RCMD); $p = 0.015$ versus myelodysplastic syndrome, unclassified (MDS-U) and 5q− syndrome].

Presence of ALIP was seen in 123 of 187 biopsies (65.8%) and 98 cases (52.4%) showed hot spot. ALIP in our series was observed more frequently in RAEB (58/64, 90.6%) than in RA and RARS (4/19, 21.1%), and there was a statistical difference ($p < 0.0001$). CD34+ clusters were observed in 74 cases (39.6%) and >5% CD34+ cells in 104 cases (55.6%). CD34+ clusters and >5% CD34+ cells were detected in 70.3% (45/64) and 84.4% (54/64) of RAEB cases, significantly more frequently than in other subgroups ($p < 0.0001$, $p < 0.0001$, respectively).

Grade 4 fibrosis was seen in nine cases (4.8%), grade 3 fibrosis in 18 cases (9.6%), grade 2 fibrosis in 48 cases (25.7%) and grade 1 fibrosis in 65 cases (34.8%). Significant fibrosis in marrow (≥2 reticulin fibrosis) was seen in 40.1% of cases, and there were no significant differences in occurrence among different subtypes.

Table 1 The clinical features of MDS patients

Feature	*n* (%)
Total	187
Age	
<60	140 (74.9%)
≥60	47 (25.1%)
Sex	
Male	108 (57.8%)
Female	79 (42.2%)
Haemoglobin (g/dL)	
≥10	70 (37.5%)
8–<10	21 (11.2%)
<8	96 (51.3%)
Neutrophils (10^9/L)	
≥0.8	162 (86.6%)
<0.8	25 (13.4%)
Platelets (10^9/L)	
≥100	86 (46.0%)
50–<100	47 (25.1%)
<50	54 (28.9%)
Transfusion dependency	
No	104 (55.6%)
Yes	83 (44.4%)
Cytogenetics	
Very good	30 (16.0%)
Good	124 (66.3%)
Intermediate	22 (11.8%)
Poor	5 (2.7%)
Very poor	6 (3.2%)
Bone marrow blasts	
0–2%	103 (55.1%)
>2–<5%	20 (10.7%)
5–10%	26 (13.9%)
>10–20%	38 (20.3%)
WHO subgroups	
RA	12 (6.4%)
RARS	7 (3.7%)
RCMD	71 (38.0%)
MDS-U	23 (12.3%)
5q− syndrome	10 (5.3%)
RAEB-I	26 (13.9%)
RAEB-II	38 (20.3%)
IPSS-R risk groups	
Very low	29 (15.5%)
Low	71 (38.0%)
Intermediate	41 (21.9%)
High	28 (15.0%)
Very high	18 (9.6%)

MDS, myelodysplastic syndrome; MDS-U, myelodysplastic syndrome, unclassified; RA, refractory anaemia; RAEB, refractory anaemia with excess blasts; RARS, refractory anaemia with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; WHO, World Health Organization.
Revised-International Prognostic Scoring System (IPSS-R) risk groups: very low, 0–1.5; low, 2.0–3.0; intermediate, 3.5–4.5; high, 5.0–6.0; very high, ≥6.0.