REVIEW

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Prognostic Value of *RUNX1* Mutations in AML: A Meta-Analysis

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Abstract

The *RUNX1 (AML1)* gene is a relatively infrequent mutational target in cases of acute myeloid leukemia (AML). Previous work indicated that *RUNX1* mutations can have pathological and prognostic implications. To evaluate prognostic value, we conducted a meta-analysis of 4 previous published works with data for survival according to *RUNX1* mutation status. Pooled hazard ratios for overall survival and disease-free survival were 1.55 (95% confidence interval (CI) = 1.11–2.15; p-value = 0.01) and 1.76 (95% CI = 1.24–2.52; p-value = 0.002), respectively, for cases positive for *RUNX1* mutations. This evidence supports clinical implications of *RUNX1* mutations in the development and progression of AML cases and points to the possibility of a distinct category within the newer WHO classification. Though it must be kept in mind that the present work was based on data extracted from observational studies, the findings suggest that the *RUNX1* status can contribute to risk-stratification and decision-making in management of AML.

Keywords: Acute myeloid leukemia- *AML1/RUNX1* mutation- prognosis- meta−analysis

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Introduction

Acute myeloid leukemia (AML) is a type of heterogeneous diseases characterized by acquired mutations, and abnormal differentiation of myeloid progenitor cells (Burnett et al., 2011; Marcucci et al., 2011). So far, evaluation of cytogenetic abnormalities provide the most significant prognostic information at diagnosis of AML (Grimwade, 2001; Mrozek et al., 2004). Moreover, acquired molecular changes have been described with prognostic significance. Understanding of leukemogenesis mechanisms mediated through the increasing number of genetic abnormalities detected in AML, to an enhancement of individual risk evaluation, and finally to the improvement of risk categorization, molecularly and targeted based therapies (Döhner et al., 2010).

For the management of AML prognostic evaluation is pivotal since therapies may be adjusted on the basis of precise assessment of outcome. Though the use of conventional cytogenetic study for risk-adaptation is broadly established (Bloomfield et al., 1998; Grimwade et al., 1998; Grimwade et al., 2001), but the AML prognosis is not adequately predictable so that supplementary prognostic factors are required for more precise assessment.

A potential prognostic genetic factor is the *AML1/RUNX1* (henceforward stated to as *RUNX1*) gene (Ito, 2008), comprising of 10 exons, is one of the commonly dysregulated genes in AML through chromosomal aberrations and somatic mutations (Osato, 2004; Yamagata et al., 2005). Adverse outcome has been associated with *RUNX1* mutation in MDS patient (Harada et al., 2004; Chen et al., 2007), but the impact of this genetic alteration in de novo AML remains less clear. On the other hand, based on all the accumulated evidence, a new provisional entity "AML with mutated *RUNX1*" was added to the classification for cases of de novo AML and it has been associated with distinct clinicopathologic features and inferior outcome (Döhner et al., 2016). However, when published in "the 2016 revision to the WHO classification of myeloid neoplasms and acute leukemia" some questions were unanswered, categories for which the WHO working groups felt there was inadequate document to recognize as distinct group at this time (Campo et al., 2011).

After these seminal observations, several studies have demonstrated the consistent and robust ability of *RUNX1* mutation status to stratify patient outcome but Gaidzik et al (Gaidzik et al., 2011) found no significant impact on relapse free survival (RFS) or overall survival (OS) within the cytogenetically normal AML (CN-AML) subset. In addition, Tang et al found a borderline adverse prognostic marker for diseae

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free survival (DFS) (Hazard ratio (HR) = 1.580; 95% confidence interval (CI) = 0.940-2.653, p-value = 0.084) in this rather homogeneous cohort, here, we performed a systematic review evaluating the prognostic value of *RUNX1* in patients with de novo AML. Moreover, due to the relatively uncommon rate of *RUNX1* mutation, it can be presumed that the limited studies might be insufficient to precisely determine the impact of the *RUNX1* mutation. Accordingly, we constructed a meta-analysis on previously published works to explore the prognostic value of *RUNX1* mutations for de novo AML. Our findings may provide the further information on the roles and its clinic implications of *RUNX1* mutation in the development and progression of AML and also further evidence to recognize as distinct category within the newer WHO classification.

Materials and Methods

Studies selection

We performed a literature review to identify studies on the prognostic value of *RUNX1* mutation status AML with the support of an experienced medical librarian. We applied no language restrictions. We searched 5 databases (Medline, PubMed, EMBASE, CENTRA and Scopus) to identify all citations to February 2017 describing the role of *RUNX1* mutation testing in predicting prognosis for AML. Ovid MEDLINE was used to design the strategy, using a combination of MeSH controlled vocabulary and text words for each concept. The following terms were used to perform the search: *AML1/RUNX1* mutation and AML AND *RUNX1* AND survival (as text words). The duplicate results were removed.

Full-length publications reporting on the prognostic value (DFS and/or OS) of *RUNX1* in patients with AML were included in the systematic review. The early search generated a total of 188 papers, 150 of which were omitted by screening of title. Summaries of the remaining 38 publications were considered, resulting in 29 of them being omitted, and leaving 9 as candidate papers. To obtain a final result on which papers could be eligible for the meta-analysis, we evaluated all the 9 articles in detail, which lead to further omission of 5 articles, because required survival-time data was not accessible for *RUNX1* mutation. Eventually, four papers (Tang et al., 2009; Gaidzik et al., 2011; Greif et al., 2012; Mendler et al., 2012) (Figure 1) to meet all the criteria stated above were considered (Table 1). Citations in the 4 articles were also followed up. Two reviewers independently reviewed all candidate relevant papers.

Study evaluation

Two reviewers independently reviewed the potential qualification of each of the abstracts produced by the comprehensive search plan. Each abstract was evaluated independently for final study inclusion.

Data extraction

Data were extracted using a standardized form to enter study participant characteristics, proportion of patients who had *RUNX1* mutation status performed, and DFS and OS for all patients. Data extraction was performed in duplicate by 2 reviewers and disagreements were solved by discussion.

Meta-analysis

We constructed meta-analyses using random-effects models (Der-Simonian Laired) to analyze pooled HR with 95% CI for DFS and OS from multivariable study results for *RUNX1* mutation status results. Heterogeneity was assessed using the between study variation (I2) from the Q statistic. A p-value of less than 0.05 defined as a statistically significant. Statistical analyses were performed using STATA ver. 14.2 software (College Station, TX, USA).

Results

As displayed in Table 1, four published studies covering about 1581 subjects were ultimately included in the meta-analysis. Two of them originated from Europe, one from Asia and another from the United States. One study reported OS, but not DFS (Greif et al., 2012). at the time of diagnosis White blood cell (WBC) counts were stated in all studies, and showed that *RUNX1*-mutation was associated with lower WBC counts (Greif et al., 2012) than cases without mutation. For patients percentages with good-risk cytogenetic group no common trend was found.

The pooled HR for DFS was 1.76 (95% CI = 1.24–2.52; p-value = 0.002) for *RUNX1* mutations, by random-effect models, proposing that the presence of either point mutation is an unfavorable prognostic marker for DFS (Figure 2). No significant heterogeneity was detected in the overall analysis (I2 = 43.2%, p-value = 0.172). Figure 3 displays the findings of a similar study for OS. The pooled HR for OS was 1.55 (95% CI = 1.11–2.15; p-value = 0.01) for *RUNX1* mutations. The heterogeneity test in the overall study exhibited no significant heterogeneity (I2 = 46.7%, p-value = 0.131) for OS either.

Discussion

The clinical course of individuals with de novo non-M3 AML is variable and difficult to predict. Recently, the last edition of the European LeukemiaNet (ELN) (Döhner

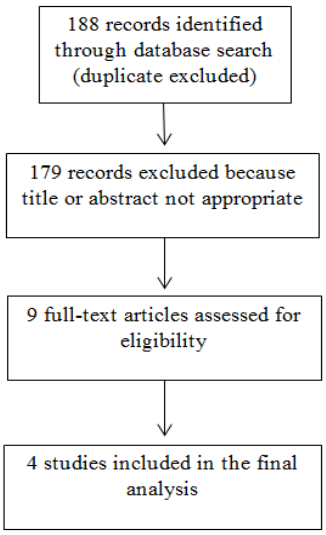

Figure 1. Literature Search Data

Figure 2. Forrest Plots of the Hazard Ratios (HRs) and 95% Confidence Intervals (CIs) for Pooled Disease Free Survival (DFS). The size of the diamonds represents the weight for the random-effect model in the meta-analysis. A HR higher than unity indicates that the presence of *RUNX1* mutation is associated with a worse prognosis.

Figure 3. Forrest Plots of the Hazard Ratios (HRs) and 95% Confidence Intervals (CIs) for Pooled Overall Survival (OS). The size of the diamonds represents the weight for the random-effect model in the meta-analysis. A HR higher than unity indicates that the presence of *RUNX1* mutation is associated with a worse prognosis.

et al., 2016) proposed a new categorization strategy for CN-AML according to the mutation of *RUNX1*. Approximately patients with RUNX1 mutation fall into the ELN Intermediate-I Group. To evaluate whether point mutations in *RUNX1* prepare supplementary prognostic significance in the context of clinical and molecular predictors, we performed meta-analysis including four published studies (a total of 1581 patients; Table 1)

The purpose of this study was to explore the prognostic effect of *RUNX1* mutations in well-annotated, relatively large cohorts of 1581 patients with de novo non-M3 AML to investigate the association between clinical outcome and *RUNX1* mutations.

The incidence of *RUNX1* mutation is relatively low (5 to 16%) and making it difficult to identify its real impact on clinical outcome. Meta-analysis is a statistical

Table 1. List of Studies Included in the Meta-Analysis and Diagnostic Characteristics According to the *RUNX1* Status in the Patients with AML and de Novo non-M3 AML

Author	RUNX1 status	Number	Age	Initial WBC	Tumor type	HR for DFS	95% CI for DFS	HR for OS	95% CI for OS
Tang	NA	234	WT: 48.0 (15-90) Mu: 62.0 (15-89)	WT: 22430 (120-627800) Mu: 15 225 (310-258900)	AML non-m3	WT: 1 Mu:1.580	Reference (0.940-2.653)	WT:1 Mu: 1.874	Reference (1.075-3.269)
Verena	WT: 831(94.1%) Mu: 53(5.9%)	884	WT: 48.1 (19-60) Mu: 48.2 (18-60)	WT: 13.4(0.9-235.0) MU: 17.6(0.2-427.0)	AML non-m3	WT: 1 Mut: 1.296	Reference (0.843- 1.992)	WT:1 Mut:1.077	Reference (0.755-1.535)
Jason	WT: 343(87.5%) Mu: 49(12.5%)	392	WT: 61 (18-83) Mu: 68 (30-81)	WT: 28.6(0.9-450.0) MU: 21 (0.9-434.1)	AML non-m3	WT: 1 Mu: 2.34	Reference (1.49-3.66)	WT:1 Mut:1.65	Reference (1.13-2.42)
Greif	NA	71	WT: 54 (27-83) Mu: 73 (54-78)	WT: 39.5 (0.1-486.0) MU: 11.70 (1.8-105.3)	AML non-m3	NA	NA	WT:1 MU: 2.51	Reference (1.1-5.8)

CI, confidence interval; HR, hazard ratio; DFS, disease free survival; Mu, mutant; NA, not available; OS, overall survival; WBC, white blood count; WT, wild type.

technique in evidence-based medicine for combining findings from several independent primary works for a specified outcome. Integrating the homogeneous works will increases statistical power, and provide a meaningful summary. The present meta-analysis revealed that the impact of *RUNX1* mutations were fairly impressive with the pooled HR of 1.76 (95% CI = 1.24–2.52; p-value = 0.002) for DFS, and 1.55 (95% CI = 1.11–2.15; p-value = 0.01) for OS. Consistent with the previous findings, *RUNX1* mutation was found to be associated with adverse prognosis.

These results support the idea that *RUNX1* plays an important role in configuration the clinical outcome of AML patients, and propose that *RUNX1* mutation can be pivotal for better risk categorization. These results are in line with other studies (Tang et al., 2009; Greif et al., 2012) who reported that *RUNX1* mutations are associated with shorter OS and RFS in homogeneously treated CN-AML patients. However, in another study, not found significant impact on RFS or OS within the CN-AML subgroup (Gaidzik et al., 2011).

Our meta-analysis has some limitations. The first limitation is that our meta-analysis was based on observational studies rather than randomized controlled trial and prospective studies. Secondly, to obtain information we used summarized information, while an individual participant data meta-analyses are regarded as the gold standard and would provide a reliable assessment of the association. The present findings must be carefully interpreted by clinicians. Thirdly, a considerable impact of heterogeneity needs to be taken into account. The existence of various level of heterogeneity among this study might be due to distribution of the intermediate and unfavorable cytogenetics, differences in treatment, which were not explored in the study. Lastly, we cannot found publication bias, but it's can also have had an undesirable impact on the precision of the analysis.

But despite these limitations, the present study revealed that *RUNX1* alterations do have a negative impact on clinical outcome for AML. These results may make it reasonable to differentiate AML with mutated *RUNX1* from AML with unmutated *RUNX1* and better therapeutic plan for AML may be obtain based on *RUNX1* mutation. Nevertheless, in prospective cohort studies the investigators confirmed relatively large cohorts of patients must be explored to make it conceivable to obtain any decisive conclusion. Our findings provide another layer on the roles and its clinic implications of *RUNX1* mutation in the development and progression of AML and also further evidence to recognize as distinct category within the newer WHO classification.

In conclusion, we provide additional evidence for the adverse effect of *RUNX1* mutations on clinical outcomes in AML patients. Our meta-analysis focused on de novo non-M3 patients. Importantly, our results that *RUNX1* mutations in AML patients are related with a dismal prognosis should aid in defining these patients as a group that could potentially benefit from alternative treatment strategies.

Conflict of interest statement

The authors declare no conflict of interest.

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