The Role of Ras Oncogene Mutations in Acute Myeloid Leukemia Patients: A Meta-analysis Based on 2502 Cases

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Abstract: Background: the ras oncogene mutations frequently occurred in acute myeloid leukemia (AML), but as a prognostic factor remains inconclusive. Methods: The databases of PubMed, Web of Science, EMBASE, and the Cochrane. 22 eligible studies were included this study and analysis was conducted by Comprehensive Meta-Analysis Version 2 software program. All eligible study’s quality assessment refers to the European Lung Cancer Party quality scale. Results: Combined analysis showed that ras oncogene mutation was a poor impact on survival in AML patients (Hazard ratios (HRs): 1.50, 1.19-1.89, \( p < 0.001 \)). Nras gene mutation was a worse survival marker in AML (HR: 1.97, 95% CI: 1.35-2.89, \( p < 0.001 \)) and Kras gene mutations was no significance (HR: 1.32, 95% CI: 0.83-2.09, \( p = 0.24 \)) by stratified analysis. In the analysis of age bracket, adults with ras gene mutation had an unfavorable survival (HR: 1.55, 95% CI: 1.19-2.21, \( p = 0.01 \)) and children harbored ras gene mutation was not significantly with prognosis (HR: 1.22, 95% CI: 0.97-1.53, \( p = 0.09 \)) in AML. Conclusions: This study indicated that AML patients was poor prognosis especially in adult group with ras oncogene mutation, in which Nras mutation, but not Kras mutation involved in guiding survival.

Keywords: Acute Myeloid Leukemia, AML, Ras, Prognostic

1. Introduction

Acute myeloid leukemia (AML) is the most frequent acute leukemia among adults [1, 2]. It is estimated that 19,950 people will be diagnosed with AML in 2016, and 10,430 patients will die of this disease [2]. In addition, approximately one third myelodysplastic syndrome will development into AML, and these transform cases have a seriously poor prognosis [2, 3]. Despite improvements in diagnosis and therapy, AML is still a disease with a very variable prognosis and a high mortality rate: 5 years survival rate is still less than 50%, and the old patients only 20% will survive 2 years after diagnosis [4].

Recently, genetic prognostic markers are recommended and considered as crucial in the development of AML and in helping therapy [2]. A variety of novel genetic markers have been identified and used to help refine prognostics groups. For instance, FLT3 internal tandem duplications (FLT3-ITD) [5], KIT [6] and IDH mutations (IDH1 and IDH2 combined) [7] have been identified as a poor prognostic marker in AML. On the contrary, NPM1 [8] and CEBPA [9] mutations grants a higher complete response rate and improves the overall survival in AML cases.

The ras oncogene as the most classical oncogene, has been proved play a key role in the tumorigenesis [10] and its family consists of three small GTP proteins (H-ras, N-ras, and K-ras). ras oncogene mutations have been generally accepted as oncogenic factor in lung [11], pancreatic [12] and colorectal cancer [13], including hematologic malignancies [14]. ras mutations frequently occurred in AML patients (Overall ras mutations: 53%; Nras in 45%; Kras in 13%), and it also be thought to lead to leukemogenesis [15]. However, ras
oncogene mutation and its prognostic role in AML patients remains inconclusive. Some studies showed that there was a poor survival in AML patients with ras gene mutation [16-18], others suggested that there was no significant prognostic relationship in AML [19-21], and there also several studies found that mutations in ras were associated with a favourable prognosis [22-24]. However, many previous studies didn’t distinguish the subtypes of ras oncogene when analysed the prognostic relationship with AML. There is a fact that different ras gene are preferentially associated with different types of human cancer. In AML patients, Nras mutation occurs more frequently than Kras mutation, whereas Hras mutations are rare. Therefore, if only Nras mutation is a prognostic fact, thus, it maybe bring bias and underestimate the risk value even error results don’t consider these differences. To test this hypothesis and clarify the prognostic correlation between ras oncogene mutation and AML patients, there is badly in need of performing a combined analysis of online studies to obtain a more accurate assessment.

2. Methods

2.1. Literature Search and Selection Reasons

A systematic literature review was performed by two independent researchers (SL and PBZ) of the PubMed, Web of Science, EMBASE and Cochrane Library databases for papers online from 1990 to 2018. The following search terms were used: (AML OR (acute myeloid leukemia) AND (ras or Kras or K-ras or Nras or N-ras or Hras or H-ras) AND (Prognosis or Prognostic)).

The literature search and eligible criteria were as below: (1) The research design was a prospective or retrospective study; (2) The study was to research the relationship between ras gene mutation and the prognosis survival in AML; (3) HRs of overall survival was reported or could be calculated from the study; (4) Studies were published in English or Chinese.

2.2. Data Extraction Data and Quality Evaluation

Two researchers (PWZ and SL) independently extracted the data from each included study: The name of first author, country of studies, published year, age of patients, sample size, test methods, and HRs estimation. Based on Steele’s methods, the quality evaluation of the methodology were conducted independently by two physicians (SL and PBZ) and given a quality score [25]. The quality score assessed the following four main dimensions: the scientific design; laboratory methodology; generalizability; and the result analysis. A maximum of 10 points was given in each category. Each dimensions score was obtained the mean from three investigators. Finally, quality scores were calculated in the form of percentages, and the value was ranged from 0 to 100%, and the higher scores indicating superior methodology.

2.3. Statistical Analysis

The p<0.05 was regarded as significant prognostic relationship for the statistical test comparing the groups with and without mutations of ras in AML patients. The p≥0.05 identified that there is no statistically significant difference between with ras mutation and without ras mutation in terms of survival in AML.

The HRs was used to evaluate the overall survival effect. The HRs was obtained using a method based on the outcomes reported in eligible articles [26, 27]. If only reported the total number of cases, the number of each group, and the logrank p value, and an approximate HRs can be calculated [26]. In addition, if the only effective data were provided in the form of survival curves, the data from Kaplan-Meier survival curves can be extracted by the Engauge Digitizer software and calculate HRs by the Parmar’s method [28]. With respect to the quality assessment, mean and standard deviation were given in each group. Differences between groups and p value were calculated by the nonparametric U test [29]. Q statistic and I² statistics was used for detecting the statistical heterogeneity within studies (If I²≤50% identified lower heterogeneity and the fixed-effects model was selected; If I²>50% identified higher heterogeneity and the random-effects model was selected) [30]. Subsequently, the publication bias was detected by the Egger’s method and observed in the form of funnel plot [31]. If publication bias was detected, then the method of Duval and Tweedie’s trim-and-fill was used to adjusted HRs [27].

3. Results

3.1. Study Selection and Characteristics

Two reviewers perused 134 studies independently and excluded 112 articles. Finally, 22 articles were selected in this meta-analysis [16, 17, 19, 21, 32-49]. Studies were published from 1990 to 2014. The details of excluding the articles were as below: 56 articles didn’t reported HRs and also couldn’t calculate HR as insufficient data, 38 studies did not related to topic, 10 articles did about therapy and 8 articles of cases of patients less than 20. The search process is shown in Figure 1.

The main features of the included articles were shown in Table 1. According to the research region distribution, 5 studies were performed in Europe [32, 34, 40-42], 6 studies in North America [19, 21, 36, 45, 46, 49], 6 studies in Asia [17, 33, 35, 38, 43, 48], 2 studies in Oceania [37, 47], 2 studies in Africa [16, 39] and 1 study in South America [18].
There were several methods to identify the mutation ras gene, 7 studies used by PCR and directly sequencing [17, 32, 33, 35-38]. 8 studies were through the PCR and Oligonucleotide hybridization methods [42-49]. 4 studies conducted by PCR and Melting curve analysis [34, 39-41]. 2 studies used PCR and Single-Strand Conformation Polymorphism [16, 21] and 1 study used PCR and High Performance Liquid Chromatography [19].

### 3.2. Quality Evaluation

The global quality score which ranged from 55.00% to 74.17%, and the mean and standard deviation were 63.66% and 5.53, respectively (Table 2). With respect to the global score, there was no statistically significant difference was identified between the 8 studies that were reported HRs [16, 19, 21, 33, 34, 36, 37, 45] and the 14 studies calculated HRs.
The Role of Ras Oncogene Mutations in Acute Myeloid Leukemia Patients: A Meta-analysis Based on 2502 Case Studies (n=22) with 417 ras mutation cases of 2502 AML patients were systematically analysed in this meta-analysis. As shown in Figure 2, the pooled HRs was 1.50 (1.19-1.89, p<0.001), with medium heterogeneity ($I^2=57.88$, $p<0.001$). The results showed that the ras oncogene mutation was an unfavourable prognostic marker associated with AML patients.

### Table 2. Results of quality score of eligible studies refer to the European Lung Cancer Working Party score.

<table>
<thead>
<tr>
<th>Studies (n)</th>
<th>Global score (mean %)</th>
<th>Design (mean)</th>
<th>Laboratory method (mean)</th>
<th>Generalizability (mean)</th>
<th>Results analysis (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All studies (N=22)</td>
<td>63.66</td>
<td>7.65</td>
<td>5.53</td>
<td>6.70</td>
<td>5.56</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>5.33</td>
<td>1.16</td>
<td>1.14</td>
<td>1.20</td>
<td>1.00</td>
</tr>
<tr>
<td>HR Evaluation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reported (8)</td>
<td>64.48</td>
<td>7.21</td>
<td>5.38</td>
<td>7.08</td>
<td>6.13</td>
</tr>
<tr>
<td>Calculated (14)</td>
<td>63.10</td>
<td>7.17</td>
<td>5.62</td>
<td>6.48</td>
<td>5.24</td>
</tr>
<tr>
<td>$P$</td>
<td>0.482</td>
<td>0.714</td>
<td>0.643</td>
<td>0.305</td>
<td>0.062</td>
</tr>
<tr>
<td>Results</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significant (6)</td>
<td>64.00</td>
<td>8.20</td>
<td>5.40</td>
<td>6.40</td>
<td>5.60</td>
</tr>
<tr>
<td>Nonsignificant (16)</td>
<td>63.48</td>
<td>7.53</td>
<td>5.56</td>
<td>6.78</td>
<td>5.55</td>
</tr>
<tr>
<td>$P$</td>
<td>0.880</td>
<td>0.319</td>
<td>0.762</td>
<td>0.649</td>
<td>0.820</td>
</tr>
<tr>
<td>Age Bracket</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Children (7)</td>
<td>63.21</td>
<td>7.38</td>
<td>5.14</td>
<td>7.05</td>
<td>5.71</td>
</tr>
<tr>
<td>Adults (13)</td>
<td>64.23</td>
<td>7.72</td>
<td>5.95</td>
<td>6.54</td>
<td>5.49</td>
</tr>
<tr>
<td>$P$</td>
<td>0.817</td>
<td>0.311</td>
<td>0.056</td>
<td>0.438</td>
<td>0.699</td>
</tr>
<tr>
<td>Test Methods</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCR-Seq (7)</td>
<td>62.26</td>
<td>7.24</td>
<td>5.05</td>
<td>7.10</td>
<td>5.52</td>
</tr>
<tr>
<td>PCR-ONH (8)</td>
<td>62.87</td>
<td>8.08</td>
<td>5.74</td>
<td>6.07</td>
<td>5.26</td>
</tr>
<tr>
<td>$P$</td>
<td>1.00</td>
<td>0.252</td>
<td>0.091</td>
<td>0.174</td>
<td>0.918</td>
</tr>
</tbody>
</table>

Abbreviations: No. of Pts=Number of patients; HR=Hazard Ratio; PCR=polymerase chain reaction, Seq=Sequencing, ONH= Oligonucleotide sequencing.

### 3.3. Meta-analysis

The overall 22 studies with 417 ras mutation cases of 2502 AML patients were systematically analysed in this meta-analysis. As shown in Figure 2, the pooled HRs was 1.50 (1.19-1.89, p<0.001), with medium heterogeneity ($I^2=57.88$, $p<0.001$). The results showed that the ras oncogene mutation was an unfavourable prognostic marker associated with AML patients.
Figure 2. Meta-analysis evaluated the prognosis of AML with ras oncogene mutation. (A) The forest plot for evaluating all included studies. (B) The funnel plot for detecting the publication bias.

Subsequently, subgroup analyses in AML groups were conducted according to HR evaluation, test methods, mutation types, study regions and the age bracket. The results were shown in Table 3.

<table>
<thead>
<tr>
<th>Mutations Types</th>
<th>Studies (n)</th>
<th>HR (95% CI)</th>
<th>P value</th>
<th>Heterogeneity</th>
<th>P for Begg (2-tailed)</th>
<th>P for Egger (2-tailed)</th>
<th>Pub. bias</th>
<th>AHR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (9)</td>
<td></td>
<td>1.97 (1.35-2.89)</td>
<td>&lt; 0.001</td>
<td>65.35</td>
<td>0.02</td>
<td>0.49</td>
<td>NO</td>
<td>1.97 (1.35-2.89)</td>
</tr>
<tr>
<td>K (5)</td>
<td></td>
<td>1.32 (0.83-2.09)</td>
<td>0.24</td>
<td>36.68</td>
<td>0.177</td>
<td>1.00</td>
<td>NO</td>
<td>1.32 (0.83-2.09)</td>
</tr>
<tr>
<td>N&amp;K (10)</td>
<td></td>
<td>1.12 (0.92-1.36)</td>
<td>0.25</td>
<td>0.63</td>
<td>0.37</td>
<td>0.36</td>
<td>NO</td>
<td>1.09 (0.91-1.32)</td>
</tr>
<tr>
<td>Study Regions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Europe (5)</td>
<td></td>
<td>1.08 (0.87-1.35)</td>
<td>0.46</td>
<td>0</td>
<td>0.658</td>
<td>0.88</td>
<td>NO</td>
<td>1.08 (0.87-1.35)</td>
</tr>
<tr>
<td>Asia (6)</td>
<td></td>
<td>1.73 (1.9-2.51)</td>
<td>0.004</td>
<td>0</td>
<td>0.88</td>
<td>0.71</td>
<td>NO</td>
<td>1.73 (1.9-2.51)</td>
</tr>
<tr>
<td>North America (6)</td>
<td></td>
<td>1.66 (0.96-2.85)</td>
<td>0.07</td>
<td>79.73</td>
<td>&lt; 0.001</td>
<td>0.45</td>
<td>NO</td>
<td>1.66 (0.96-2.85)</td>
</tr>
<tr>
<td>Oceania (2)</td>
<td></td>
<td>0.87 (0.45-1.67)</td>
<td>0.68</td>
<td>0</td>
<td>0.84</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Africa (2)</td>
<td></td>
<td>2.54 (1.38-4.69)</td>
<td>0.003</td>
<td>24.10</td>
<td>0.25</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>South America (1)</td>
<td></td>
<td>3.08 (1.51-6.29)</td>
<td>0.002</td>
<td>0</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: HR=Hazard ratio; CI=confidence interval; Pub.bias=publication bias, AHR=Adjust HR, Seq=Sequencing, PCR= polymerase chain reaction, ONH=Oligonucleotide sequencing, SSCP= Single-Strand Conformation Polymorphism, HPLC= High Performance Liquid Chromatography.
8 studies reported the HRs identified that there were significantly prognostic relationship in AML with ras oncogene mutation, and the pooled HR was 1.02 (95% CI: 0.83-1.25, \( p=0.85 \)) (see Supplementary Figure A1 A online). For 14 studies in which HRs were not directly provided and were calculated from the original articles based on published data and figures, and yielding a combined HR of 1.56 (95% CI: 1.12-2.15, \( p=0.01 \)) (See Supplementary Figure A1 B online).

Subgroup in test methods, we found there was statistically significant prognostic relationship in AML patients with ras oncogene mutation by the PCR-direct sequencing method (PCR-Seq), PCR-oligonucleotide sequencing (PCR-ONH) and PCR-Single Strand Conformation Polymorphism (PCR-SSCP), and yielding the combined HR were 1.73 (95% CI 1.19-2.51, \( p=0.001 \)) and 2.63 (95% CI: 1.58-4.39, \( p=0.001 \)), respectively (See Supplementary Figure A2 A-C online).

Based on the different study regions, a statistically significant prognostic relationship in AML with ras gene mutations was found in Asia and Africa region, and the pooled HRs were 1.73 (95% CI 1.19-2.51, \( p=0.001 \)) and 2.54 (95% CI: 1.38-4.69, \( p=0.003 \)), respectively. However, no statistically significant association was found in Europe (pooled HR: 1.08, 95% CI: 0.87-1.35, \( p=0.46 \)) and North America (pooled HR: 1.66, 95% CI: 0.96-2.85, \( p=0.07 \)) (See Supplementary Figure A3 A-C online).

When analyzed different types of mutation ras gene, we found a lower prognostic value in Nras mutation (pooled HR: 1.97, 95%CI: 1.35-2.89, \( p<0.001 \)), but not in Kras mutation (pooled HR: 1.32, 95% CI: 0.83-2.09, \( p=0.24 \)). In addition, 11 studies which didn’t distinguish Nras and Kras, yielding a combined HRs of 1.09 (95% CI: 0.91-1.32, \( p=0.35 \)) (See Supplementary Figure A4 A-C online).

Finally, according to the subgroup of age bracket, we found that there was a worse prognostic value in adults with ras gene mutations, and with HRs was 1.55 (95% CI: 1.19-2.21, \( p=0.01 \)), but not in children (pooled HR: 1.22, 95% CI: 0.97-1.53, \( p=0.09 \)) in children (See Supplementary Figure A5 A-B online).

4. Discussions

A high frequently ras gene mutations were found in AML patients. However, ras oncogene mutation and its prognostic effect in AML remains uncertain. In this meta-analysis, 22 eligible studies were included and the results showed that ras oncogene mutation was a worse prognostic marker associated with AML patients (HR: 1.50, 95% CI: 1.19-1.89, \( p<0.001 \)).

To further study the correlation between ras gene mutation and AML, and detect heterogeneity, we conducted subgroup analysis based on HRs estimation, test methods, mutation types, study regions, and age bracket. Quality assessment showed that there was no statistically significant difference in each subgroups and permitted us to conduct a quantitative pooled meta-analysis. According to the subgroup outcomes, there were some interesting and important results were found. When analyzed the N, K-ras gene mutation and its prognostic effect in AML patients, only Nras gene mutation was a poor prognostic factor but not Kras. The results are in accordance with the fact that Nras mutation are predominantly in AML. Mulligan et al [50] studied the difference between Nras and Kras mutations in myeloma, and found only mutation of Nras significantly decrease myeloma susceptibility to single-agent bortezomib treatment, but not Kras. In addition, mice model have provided a lot of evidences about Nras gene mutation and its role in leukemogenesis. Such as, Parikh et al [51] used an improved mouse bone marrow transduction and trans-plantation model and found that oncogenic Nras rapidly and efficiently induced AML like disease in mice. Li et al [52] generated Lox-STOP-Lox (LSL)-NrasG12D mice and showed mutant Nras alters the distribution of hematopoietic stem and progenitor cell in hematopoietic tissues, in addition, the mice die of aggressive myeloproliferative disorder by 4 months of age. Cuiffo et al [53] used the mice model and found palmitoylation was essential for leukemogenesis by oncogenic Nras.

In subgroup of study regions, only study regions in Asia and Africa showed that an unfavorable survival for AML patients with ras mutation, but not in Europe and North America regions, limited researches were inadequate to get a conclusion that Asia and Africa people were susceptible population.

Finally, subgroup analysis the age bracket, we found that there was a very low survival effect in adults group with ras mutation (HR: 1.55, 95% CI: 1.09-2.21, \( p=0.01 \)), but not in children group (HR: 1.22, 95%CI: 0.97-1.53, \( p=0.10 \)). According to above results, the age factor may be play an important factor which influences the prognostic analysis. Recently, a study about “Changes in Cytogenetics and Molecular Genetics in Acute Myeloid Leukemia from Childhood to Adult Age Groups” was conducted by Creutzig et al, and found a significant decrease in the proportion of favorable cytogenetic subtypes and an increase in unfavorable cytogenetics were observed with increasing age [54]. Therefore, if the published studies do not consider age factor and it may underestimate the prognostic value, even obtained a negative result [55-58].

There were several limitations in this meta-analysis. Firstly, the outcomes were calculated from online articles, and the literature search could not cover all published articles. Secondly, only studies which wrote in English were selected this study. This limitation is comes from the fact that the positive results that are prefer to publish in English, but the negative outcomes frequently published by native languages [49]. Finally, most of included studies are based on univariate analysis, and it exists many confounding factors, such as age bracket, gender and race.

5. Conclusion

In conclusion, this study indicated that AML patients was poor prognosis especially in adult group with ras oncogene mutation, in which Nras mutation, but not Kras mutation...
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involved in guiding survival. *Nras* oncogene mutation can be as a poor prognostic marker in AML patients, especially in adults. However, data are insufficient to decide its role in AML. The results still need to be confirmed by many multivariate analysis studies.

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**Disclosure**

The authors declare no conflict of interest.

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**Appendix**

![Figure A1](image.png)

*Figure A1.* The pooled Meta-analysis evaluated the prognosis of AML with ras oncogene mutation based on HR evaluation. (A) The combined HRs based on reported HRs studies. (B) The combined HRs based on calculated HRs studies.
Figure A2. The combined Meta-analysis evaluated the prognosis of AML with ras oncogene mutation based on test methods. (A) The combined HRs based on PCR-Seq method. (B) The combined HRs based on PCR-ONH method. (C) The combined HRs based on PCR-MCA method.
Figure A3. Meta-analysis evaluated the prognosis of AML with ras oncogene mutation based on study regions. (A) The combined HRs based on Europe region studies. (B) The combined HRs based on Asia region studies. (C) The combined HRs based on North America studies.
Figure A4. Meta-analysis evaluated the prognosis of AML with ras oncogene mutation based on types of mutation. (A) The combined HRs based on Nras oncogene mutation. (B) The combined HRs based on Kras oncogene mutation.
Figure A5. Meta-analysis evaluated the prognosis of AML with ras oncogene mutation based on age bracket. (A) The combined HRs based on adults group. (B) The combined HRs based on children group.

### References


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