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RESEARCH ARTICLE **OPEN ACCESS**

MODIFICATION OF THE FLT3 MUTATIONAL STATUS BETWEEN DIAGNOSIS AND RELAPSED/REFRACTORY AML: A META-ANALYSIS.

Silva, LT[1],*., Campos, CCAP[1]., Salvino, M.A.[1]

Federal University of Bahia, Salvador, Bahia

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ABSTRACT

Acute myeloid leukemia (AML) is a hematologic cancer with a high mortality and the FLT3 mutations are recognized as one of the most important alteration associated with AML and are related with a poor prognosis. Some studies have revealed that at diagnosis, patients with AML with FLT3 can maintain the alteration in the relapse or refractory disease, although this is not always the rule. This report is a meta-analysis review about the genetic change of FLT3 mutation in the relapsed/refractory AML scenario and if this what differs from findings at diagnosis in the same patients. A search in June/2020 was performed in PUBMED to identify studies with the terms FLT3 (title/abstract) AND "Acute Myeloid Leukemia" without a restrict date. Six articles described mutation status in both FLT3 alterations. The total of 272 patients with relapse and refractory AML were analyzed, 74 (27%) were positive for FLT3-ITD at diagnosis and 15 (5%) for FLT3-TKD. The change in mutation status occurred in 17% of patients. Mutation status change from negative to FLT3-ITD occurred in 7% of patients, FLT3-ITD to negative in 5%. From those that were FLT3-ITD positive 60 remained positive in the relapse. Our data demonstrated that 41% of patients were positive for FLT3 mutation in some point of the disease. Since one in six patients had their mutational status change in the study, reassessment should be mandatory in any case of relapse and refractoriness. If this is related with worst prognostic more studies are needed to elucidate.

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INTRODUCTION

Acute myeloid leukemia (AML) is a hematologic cancer with a high mortality and nowadays hasa cure rate of less than 50%[1]. In the past years, with the advance in research, the class III receptor tyrosine kinase Fms-like tyrosine kinase 3 (FLT3) was identified as a protein with an important role in hematopoiesis and that mutation in FLT3 gene is present in approximately 20-30% of AML patients[2-4]. The FLT3 mutations are now recognized as one of the most important alteration associated with AML and are related with a poor prognosis[5,6]. Two distinct types of FLT3-activation mutations have been described. The FLT3 internal tandem duplication (FLT3-ITD) that occurs in the juxtamembrane (JM) domain and FLT3 tyrosine kinase domain (FLT3-TKD) that occurs in a domain referred to as the activation loop of FLT3. Those alterations are related with cell proliferation. The FLT3-ITD is more common in AML, responsible for 25% of cases with AML, while FLT3-TKD is present in 5-10% of cases[7].However, the FLT3-TKD does not have well defined prognostic impact in AML. Some studies have revealed that at diagnosis, patients with AML with FLT3 can maintain the alteration in the relapse or refractory disease, although this is not always the rule. Some patients have different molecular profile in the relapse or refractory diseases, with acquisition or loss of new mutations[8]. The evolution of FLT3 is an important information for the clinical management because it can be related to clonal selection of the AML cells or associated with a different outcome in the relapse/refractory scenario. Associated with this, to know the incidence of FLT3 new mutations in relapse/refractory AML or the loss of this mutation in this situation have an implication in the future treatment since we have new therapeutic options with FLT3 inhibitors. Thus, this report is a meta-analysis review about the genetic change of FLT3 mutation in the relapsed/refractory AML scenario and if this what differs from findings at diagnosis in the same patients.

Corresponding author:** ***Silva, LT.,
Federal University of Bahia, Salvador, Bahia.

MATERIALS AND METHODS

Search Strategy: A search in June/2020 was performed in PUBMED to identify studies with the terms FLT3(title/abstract) AND "Acute Myeloid Leukemia" without a restrict date. Then, the papers were select based in title and abstract. Inclusion criteria were articles with acute myeloid leukemia in adult, language in English, Spanish or Portuguese. Exclusion criteria were studies in children and acute promyelocytic anemia. One author searched for the articles (LT). The selection of articles was done by reading the title and abstract. Then, the bibliography of each article was reviewed to search for articles that may be missed during the process.

Data Abstraction: Data were collected from retrospective or prospective studies which patients with relapse and refractory AML had the information about FLT3-ITD or FLT-TKD in the diagnosis and follow-up, looking for changes in mutation status. The patient mutation status was considered change if samples from the same patient were analyzed at diagnosis and relapse or refractoriness. The flow diagram of study inclusion is represented in Figure 1. Data elements abstracted included study design, population, FLT3 mutation status and type of detection method abstracted included study design, population, FLT3 mutation status and type of detection method.

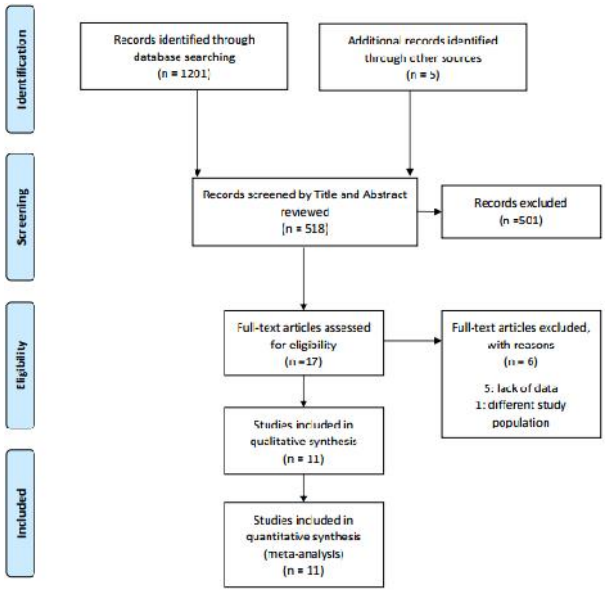

Figure 1 Flowchart of article selection for systematic review.

RESULTS

Was found 1201 papers in the initial research which was reduced to 513 articles by reading the title and abstract. From those articles 12 were eligible for full text reading. Through the bibliographic review of these articles we found 5 more articles eligible for full text reading. From eligible papers, five were excluded due to lack of data about study population and FLT3 status information, one study was excluded due a different study population selection. All the studies used PCR as the method of choice to evaluate FLT3 mutational status. Six studies evaluate FLT3 ITD and TKD, four evaluate only FLT3-ITD mutation and one only FLT3-TKD mutationas described in Table 1.

We separated the data to analyze one group for the articles that FLT3-ITD and FLT3-TKD information were available in the same population, and another two subgroups to analyze only FLT3-ITD or FLT3-TKD information status. Six articles described mutation status in both FLT3 alterations. The total of 272 patients with relapse and refractory AML were analyzed in these studies, with 74 (27%) were positive for FLT3-ITD at diagnosis and 15 (5%) for FLT3-TKD. In the first relapse or refractoriness, 81 (29%) were FLT3-ITD positive and 11 (4%) FLT3-TKD positive. The change in mutation status occurred in 47 (17%) of patients, 3 patients of these change from FLT3-TKD to FLT3-ITD mutation. There were 3 works that had patients with acute promyelocytic leukemia, a total of 10 patients. In one work (Cloos et al) 42 children were included in the study, representing 15% of the total population.

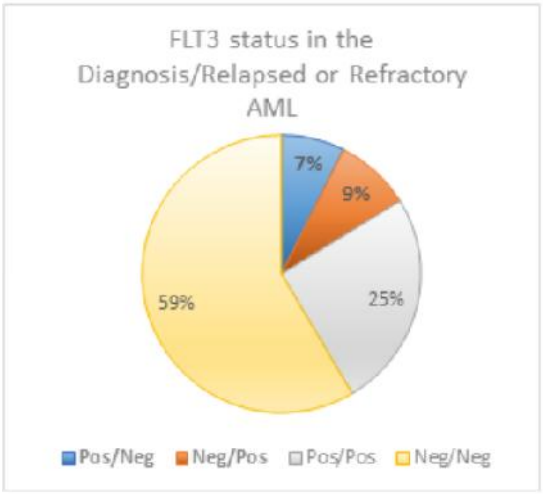

Figure 2. FLT3 status in diagnosis and R/R AML

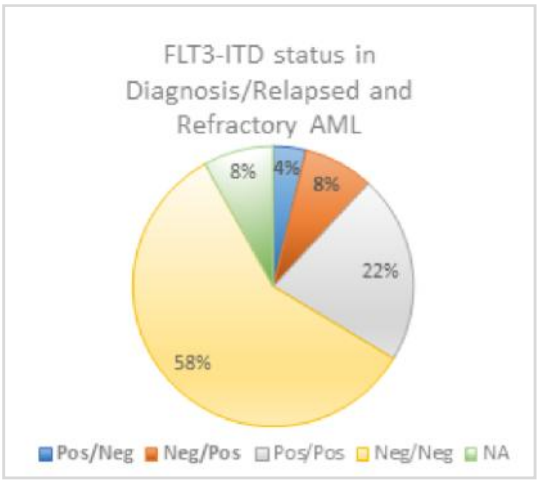

Figure 3. FLT3-ITD status in diagnosis and R/R AML

Mutation status change from negative to FLT3-ITD occurred in 20 (7%) patients, FLT3-ITD to negative in 14 (5%). From those that were FLT3-ITD positive 60 (22%) remained positive in the relapse. In patients with FLT3-TKD, change in mutation status to positive to negative occurred in 6 (2%) of the patients, 4 (1%) were negative and gained the mutation, 6 (2%) maintained positive in diagnosis and relapse. 159 (58%) were negative in all the disease course. The changes in FLT3 status are shown in Figure 2.

Table 1. Articles selected for the analysis.

Articles	Publication Year	Patients Number	Detection Method	Mutation Evaluated
Kottaridis P *et al*[9]	2002	44	PCR	FLT3-ITD and FLT3-TKD
Tiesmeier J. *et al*[10]	2004	31	PCR	FLT3-ITD and FLT3-TKD
Suzuki T *et al*[11]	2005	39	PCR	FLT3-ITD and FLT3-TKD
Cloos J *et al*[12]	2006	80	PCR	FLT3-ITD and FLT3-TKD
Palmisano M. *et al*[13]	2007	28	PCR	FLT3-ITD and FLT3-TKD
McCormick SR *et al*[14]	2010	50	PCR	FLT3-ITD and FLT3-TKD
Nakano Y. *et al*[15]	1999	28	PCR	FLT3-ITD
Shih L. *et al*[8]	2002	108	PCR	FLT3-ITD
Schnittger S. *et al*[16]	2004	97	PCR	FLT3-ITD
Nazha A. *et al*[17]	2012	102	PCR	FLT3-ITD
Shih L. *et al*[18]	2004	120	PCR	FLT3-TKD

Table 2. Studies that analyzed FTL3-ITD and FLT3-TKD and their mutational characteristics

Articles	Year	N	FLT3-ITD at Diagnosis	FLT3-TKD at Diagnosis	FLT3-ITD+/FLT 3 neg	FLT3-ITD+/FLT 3-ITD+	FLT3-ITD+/FLT 3-TKD+	FLT3 neg/FLT3-ITD+	FLT3 neg/FLT3 neg	FLT3-TKD+/FL T3 neg	FLT3-TKD+/FL T3-TKD+	FLT3-TKD+/FL T3-ITD+	FLT3 neg/FLT3 -TKD+
Kottaridis P, *et al*[9]	2002	44	18	2	5	13	0	2	20	0	2	0	2
Tiesmeier J, *et al*[10]	2004	31	7	1	1	6	0	1	22	1	0	0	0
Suzuki T, *et al*[11]	2005	39	11	0	1	10	0	4	24	0	0	0	0
Cloos J *et al*[12]	2006	80	21	1	4	17	0	5	53	1	0	0	0
Palmisano M., *et al*[13]	2007	28	6	6	1	5	0	2	12	1	4	1	2
McCormick SR, *et al*[14]	2010	50	11	5	1	10	0	4	30	3	0	2	0
Total (%)		272	74 (27%)	15 (5%)	14 (5%)	60(22%)	0 (0%)	20 (7%)	159 (58%)	6 (2%)	6 (2%)	3 (1%)	4 (1%)

The details about the mutational status in that population is described in Table 2. We extracted information about FLT3-ITD mutation from those articles and join with other works that analyzed only FLT-ITD mutation. A total of 607 patients were studied at diagnosis and relapsed, 30% were FLT3-ITD positive in relapse as demonstrated in Figure 3. Samples from 49 patients were not possible to be evaluated. Change in mutational status occurred in 12%. Patients with TKD positivity were considered as FLT3-ITD negative in this analysis. When we evaluate only TKD mutation considering FLT3-ITD as negative (Figure 4), 392 patients had their mutational status available. 89% were negative in diagnosis and relapsed. 31 (8%) patients had their status changed when only TKD mutation is considered.

DISCUSSION

Here we described FLT3 mutations status in paired samples from diagnosis to relapsed/refractory AML. Our data demonstrate that 41% of patients were positive for FLT3 mutation in some point of the disease in the study population. One in six patients had their mutational status altered. The change in mutational profile can be explained by the selection of subclones in AML.

Some patients could have small clones with mutation cells that were below the limit of the detection by assay and proliferated after the treatment. Other hypothesis is that a *de novo* mutation was acquired during the disease course, suggested by alterations in cytogenetic and cytomorphology. Some studies indicate that the gain of FTL3 mutations at relapse AML confer a poor prognosis as shown by Warren M, et al[19], with results comparable with patients with FLT3 Positive/Positive after first relapse.

From the population in the study, 7% were FLT3 positive/negative which could represent the loss of the mutation post chemotherapy and a selection of a new clone originating the relapse disease. The loss of FLT3 mutation seems to be related with better prognosis then FLT3 positivity at the relapse.[19] The method for FLT3 mutation detection in all studies was Polymerase Chain Reaction (PCR) using DNA or RNA.

This method is the most used within clinical practice. More sensitive tests could detect small clones that are below the limit detection of current method.[20,21] FLT3-ITD is more common than FLT-TKD both in diagnosis and in relapse. When only FLT3-ITD is considered the percentage of positive/negative and negative/positive is similar. But 8% of those patients did not have pared samples to evaluate mutational status. FLT3-TKD differs from other groups. Only 11% had positivity for FLT3-TKD in some point of the disease, while FLT3-ITD was present in 34%. The information about FLT3 mutation is particularly important because not only seems to be a prognostic value in patients with relapse/refractory disease but also because the patient can benefit from a novel therapeutic treatment with FLT3 inhibitors. New FLT3 inhibitors have emerged with encouraging results.[22,23] So, every patient should have an evaluation of molecular mutation in the search for acquisition or loss in relapse. A weakness of the study was the participation of only one investigator in the research of titles and abstracts which may have led to missing of some relevant article. Some articles were excluded duo lack of information that was necessary for our study. In one article the pediatric population was included which represented 15% of the population. AML in pediatric patients has some particularities and was not the focus of the study. Since one in six patients had their mutational status change in the study, reassessment should be mandatory in any case of relapse and refractoriness.

Those changes can be related with a small clone already present in the diagnosis or a new mutation acquisition related with a different AML characteristic. If this is related with worst prognostic more studies are needed to elucidate, and the development of more sensitive methods could help to find small clones already present in AML at diagnosis.

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