Analysis of C-kit Expression on Leukemic Cells Using Flow Cytometry

Thesis
Submitted for partial fulfillment for
Master Degree In
Clinical and Chemical Pathology

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Introduction:

The c-kit receptor (CD117), the product of c-kit proto-oncogene is a transmembrane tyrosine kinase receptor that is thought to play an important role in hematopoiesis **(D'Arena et al; 1998).**

Up to 4% of normal bone marrow mononuclear cells including stem cells, committed progenitor cells and mast cells bear the CD117 antigen **(Smith et al; 1994).**

In addition, CD117 is expressed by the primitive CD34 hematopoietic stem cells and is also demonstrated on the blasts of 30-100% of acute myeloid leukemia (AML) cases but rarely on lymphoblasts. Therefore, several investigators have used CD117 expression to exclude lymphoblastic origin of blasts **(Uckan et al; 2000).**

Moreover, **Nomdedèu et al (1991)** found that CD117 expression yielded a higher score in the system currently applied for the diagnosis of biphenotypic acute leukemia (BAL), as its myeloid specificity is grater than that of CD13 and CD33.

CD117 marker analysis can be performed by flow cytometric or immunohistochemical techniques. **Dunphy et al (2001)** compared the results of CD117

analysis by flow cytometry and immunohistochemical techniques, and concluded that it is better detected by flow cytometry.

Aim of the work:

Assessment of the reliability of CD117 marker in the differential diagnosis of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL), and to prove its value to br included on a routine basis for immunophenotypic diagnosis of acute leukemias.

Patients and methods:

An adequate number of patients diagnosed as acute leukemia (AML and ALL) will be subjected to:

- Full history (through clinical examination).
- Complet blood picture (CBC).
- Bone marrow aspiration.
- Immunophenotyping (IPT).
- Analysis of CD117 expression by flow cytometry.

References:

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List of abbreviations

ALL : Acute lmphoblastic leukemia
AML : Acute myeloid leukemia
APL : Acute promyeloctic leukemia
BAL : Biphenotypic acute leukemia
BAL : Biphenotypic acute leukemia
BFU-E : Burst forming unit-Erythroid
BM : Bone marrow
CBF : Core binding factor
CD : Cluster of differentiation
CFU-E : Colony forming unit- Erythroid
CFU-GEMM :Colony forming unit- Granulocyte-Erythroid- Macrophage-Megakaryocyte
CFU-GM : Colony forming unit-Granulocyte-Macrophage
CFU-Meg : Colony forming unit-Megakaryocyte
DIC : Disseminated intravascular coagulation
E.M : Electron microscope
EGIL : European group for the immunological classification of Leukemias
FAB : French-American-British
FDPs : Fibrin degradation products
FITC : Fluorescin isothiocyanate
EGIL : European Group for the Immunological Classification of Leukemia
HLA : Human leukocytic antigen
HMC-1 : Human mast cell line

Ig : Immunoglobulin
JMD : Juxta membrane domain
MDS : Myelodysplastic syndrome
McABs : Monoclonal antibodies
MPO : Myeloperoxidase
MYH : Myosin heavy chain
NOS : Not otherwise specified
PAS : periodic acid schiff
PBS : phosphate buffer saline
SCF : Stem cell factor
SIg : Surface immunogloblin
TCR : T-cell receptor
TdT : Terminal deoxynucleotide transferase
TM4SF : Transmembrane 4 superfamily
TSG : Tumor suppressor gene
WBC : White blood cell count
WHO : World health organization

CONCLUSION

Our study showed that CD117 positive expression was highly associated with acute myeloblastic leukemia, than in acute lymphoblastic leukemia, though it had no correlation with the FAB-subtypes, the myeloid markers and CD34. Also, CD117 expression had no significant correlation concerning both hematological and clinical data.

CD117 had higher specificity than CD13 and was equal to CD33; it had the lowest sensitivity than MPO, CD13 and CD33.

Recommendations

- Monoclonal antibodies recognizing the c-kit (CD117) should be routinely used in the panel of markers for the diagnosis of acute myeloid leukemia.
- C-kit marker should score for the myeloid lineage, at least one point in the score system for diagnosis of BAL.
- Further studies should be carried out to elucidate possible relation between CD117 expression and the clinical outcome of the patients.

Discussion

In this study, we assessed the reliability of CD117 in the diagnosis and in differentiation between AML and ALL. We also compared its diagnostic accuracy with other myeloid markers and with CD34 expression.

The patients had been immunophenotyped by FCM analysis, using an appropriate panel of monoclonal antibodies, as FCM is the most rapid and reliable method for distinguishing different lineage involvement. **(Jennings et al; 1997)**

Flow cytometric analysis was done on 43 cases of acute leukemia, divided into three groups; 20 cases of AML, 20 cases ALL, and 3 cases biphenotypic acute leukemia (BAL).

The results of the present study showed positive expression of CD117 in 15 cases of AML group (75%), which is similar to the results reported by **Drexler et al (1991)**, who found that 28/35 (80%) AML patients were CD117 positive.

While **Nomdedèu et al (1999)** found that 50/55 (90.9%) AML patients were c-kit positive. **Muroi et al (1996)** found that c-kit expression ranged from 23-87% of AML cases. On the other hand, in a study by **Bene et al (1998)**, done on 1937 AML patients, it was found that 741 patients (67%) were c-kit positive. Also **Hans et al (2002)** reported that CD117 was expressed in 42/66 (64%) of de novo AML patients.

On the other hand, only 3/20 (15%) ALL patients expressed CD117 marker. Out of the three cases two were

T-ALL and one was B-ALL. These data are similar to those obtained by **Muroi et al (1998)**, who reported that 2/13 (15.4%) B-ALL patients were c-kit positive. **Bene et al (1998)**, reported that 34/819 ALL patients (4%), expressed c-kit, and of these, two thirds (22.6%) were T-lineage ALL, from which 11% were c-kit positive, and the other third (11.3%) was B lineage ALL, from which 2% were c-kit positive.

Nomdedèu et al (1999) reported that from 26 cases of ALL, three T lineage ALL cases were negative for CD117, and with the exception of four cases, all the remaining B lineage ALL were CD117 negative.

On the other hand, **Cascavilla et al (1998)** reported on a study of 53 patients with ALL, that surface expression of CD117 was never observed, whether on B or T lineages.

Muroi et al (1998) concluded that c-kit expression is useful to rule out the diagnosis of B-ALL.

Also**, Bene et al (1998)** in his study established the high specificity of CD117 marker to the myeloid lineage and thus, support its value as a diagnostic reagent in the characterization of acute leukemias.

On the present work CD117 expression was not correlated to FAB subtype, which is consistent with **Dunphy et al (2001)**, who reported on a study on 29 AML patients, that there was no correlation of FAB subtype and CD117 expression. Similarly **Cascavilla et al (1998)** did not note a significant correlation between FAB classification and CD117 in a study done on 82 AML patients.

Cascavilla et al (1998) concluded that CD117 antigen showed a high specificity for AML, independently of FAB classification.

Expectedly, regarding the expression of c-kit in biphenotypic acute leukemia, the three cases included in our study were positive for CD117.

Bene et al (1998) stated that due to its now well-established specificity for the myeloid lineage, the c-kit marker should score for the myeloid lineage at least one point in the score system, for diagnosis of biphenotypic acute leukemia.

In our study we examined the association of CD117 with myeloid markers CD33, CD13, MPOX, and no significant correlation was found as P value was, (0.351, 0.741, 0.741) respectively.

Newell et al (2003), reported a case of M2 subtype, where leukemic blasts expressed CD117, but did not show definitive expression of the myeloid markers CD33 or CD13.

Our study showed that 50% of the patients with AML expressed both CD34 and CD117antigens, while only 10.5% of the patients with ALL expressed both antigens.

Nomdedèu et al (1999) reported in a study that 32/50 AML cases (64%) co-expressed CD34 on a variable proportion of CD117 positive cells, whereas 18 (36%) positive CD117 patients did not express CD34.

On the other hand, **Bene et al (1998)** reported that there was no correlation between expression of CD34 and c-kit in AML, as 24% of cases expressed one of the two

antigens, and 76% of cases either expressed, or did not express the two antigens. While **Cascavilla et al (1998)** found a direct correlation between CD34 and CD117.

Regarding CD117 sensitivity to detect myeloid involvement our present study showed that CD117 sensitivity was 75%, which was lower than the myeloid markers CD33, MPO, CD13 (95%, 90%, and 90% respectively). This is in consistent with **Nomdedèu et al (1999)**, who found that CD117 sensitivity was 91%, which is less than CD13, CD33 (92%, 93% respectively). The specificity of CD117 in our study was (85%) which was equal to CD33, higher than CD13 (65%), but lower than MPO (100%). While Nomdedèu **et al (1999)**, found that CD117 specificity was (86%) which was higher than CD13 and CD33 (36%, 44% respectively).

Chen et al (2005), also reported in a study done on 184 patients that the sensitivity of CD13, CD33 was 95.5%, 91.2% respectively, and that of MPO was low (69.1%), while CD117 sensitivity was 88.2%. The specificity of CD13, CD33 was 72.5, 62.2% respectively. On the other hand the specificity of MPO and CD117 were similar to each other (100%).

Also, **Drexler et al (1991)** reported that the sensitivity of c-kit to detect AML was slightly lower than CD13 and CD33 but its specificity was significantly higher.

Our present work showed that the positive predictive value (PPV) of CD117 was (83.3%) and the negative predictive value (NPV) was (80.9%), which was found to be close to the results of **Nomdedèu et al (1999)**, as PPV was (95%) and NPV was (77%).

In the present work the myeloid markers MPO, CD33, and CD13 had PPV of 100%, 90.4% and72% respectively, while the NPV for the same markers was 90.9%, 94.4% and 86.6% respectively.

Similarly, **Nomdedèu et al (1999)** reported that PPV for CD33 expression was (82%); NPV was (71%). While for CD13 expression, PPV was (79%) and NPV was (63%).

The diagnostic accuracy of MPO (95%) and CD33 (90%) was higher than that of CD117 (80%) and CD13 (77.5%)

Finally, c-kit (CD117) seems to be an additional reliable marker, which in combination with other antigens might facilitate the immunologic characterization of acute leukemia, especially those of myeloid origin. Though it is less sensitive than MPO, CD33, CD13, its specificity is clearly superior to those of CD33 and CD13.