RESEARCH ARTICLE

VALUABLE AND COST EFFECTIVE COMBINATION OF CD123 AND CD22 FOR BASOPHIL DISCRIMINATION

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ABSTRACT: Although the phenotypic features of basophils have been highlighted in literatures, yet some labs generate confusion while discriminating blasts from basophil gate in flow cytometry analysis. This is sometimes due to the overlap of different population on CD45 side scatter plot, and in other situations due to lack of markers which highlight basophils due to financial jeopardy. **Aim of the study**: Selection of a proper combination of markers with unique expression on basophils to clearly highlight basophils especially in under-resourced labs with limited budget. **Methods:** Comprehensive basophil phenotyping applied on 40newly diagnosed cases of CML and 20 cases of regenerating marrow post chemotherapy for Precursor B lymphoblastic leukemia with no evidence of abnormal cellsas control. Flow cytometry markers used were CD45, CD38, CD71, CD33, CD22, CD123, HLA-DR, CD34, CD117, CD3, CD4, CD8, CD56, CD11b, HLA-DR and CD15. **Results**: Basophils from patients and control samples were clearly discriminated from blasts and lymphocytes by using CD123 and CD22, a unique pattern of expression with perfect back-gating on CD45 side scatter plot which proved the discrimination. **Conclusion**: CD123 and CD22 will clearly discriminate basophils from different types of population which may occupy the window area as myeloblast, precursor B cells (hematogones), abnormal B cells (lymphoblasts) and mature B cells. This combination should therefore be part of the screening panel used as first line along with CD45in labs with limited budget.

KEY WORDS: Basophil, CML, CD123, CD22.

INTRODUCTION:

Flow Cytometry has clearly and quickly emerged to be the most helpful technique in hematology lab with predominant role in the diagnose of hemato-logical and non hematological malignancy. Also it can be used for accurate enumeration of bone marrow cells in an objective analytical method[1]. Basophil is a unique type of granular myeloid offspring; its granules have high solubility in water, which results in its location in de-granulated cells with low side scatter properties, this criterion make it stand close to lymphocytes and occupy part of blasts window in

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CD45 side scattered plot. Basophilia is manifested in different condition of benign and malignant diseases. Myeloproliferative malignancies, typically chronic myeloid leukemia (CML) characterize by basophilia, as well as polycythemia rubravera, myelofibrosis and rarely essential thrombocythemia. Basophilia is also seen in benign condition such as hypothyroidism, hypersensitivity reaction, autoimmune disease as rheumatoid arthritis and ulcerative colitis.[2]

Many markers have been used to highlight basophil in both normal and diseased condition as CD9,CD13,CD22,CD25,CD33,CD36,CD38,CD45 and CD123,294 and 69 [3]

CD22 is one of the Pan B cell markers, however it is not restricted to that lineage as it has been reported as one of basophil marker with a different pattern of expression. In spite of being bright on B cells,it tends to be dimmer on Basophiles,this difference in pattern related to the structure difference of the isoform ligand. [4]

CD123 is an Interleukin-3 receptor alpha ,it is highly expressed on plasmacytoid dendritic cells and basophils and to lesser degree on monocytes, eosinophil, myeloid and dendritic. It is also expressed on myeloblasts , mature subpopulation of hematogones and mature B cell in certain malignancy such as Hairy cell leukemia.[5,6]

CD25 is an interleukin 2 receptor alpha, its dim expression on basophils had been reported. Its expression on malignancy myeloidprecursor cellsis associated with bad prognostic molecular markers such as FLT3 and DNMT genes.[7]

CD117 is a c- kit marker heavily expressed on Mast cells and also detected on the surface normal myeloblast and promyelocytes, however its expression on normal basophil has not been detected.[8]

Although many markers have been proven to be expressed on basophil, yet a cost effective specific combination has not been discussed for accurate robust identification. This combination could be used in every panel used for identification of WBC, leukemia cells, MRD cells. This combination will discriminate Basophil from myeloblast and also B cell population.

SUBJECT AND METHODS:

40 newly diagnosed cases labeled as CML,18 female and 22 males age range from 22 to 76 years old, 38 cases were in chronic phase of CML and 2 cases were labeled as accelerated phase based on the WHO criteria published 2008. The CML cases were collected over a 4 years period, from January 2014 to December 2018. 20regenerating phenotypically normal marrows with no residual abnormal cells were assessed for Basophil population as control. 12 pediatric patients (less than 16 years) and 8 adult patient (more than 16 years old). The study was approved by the research ethical committee of Al Qassimgovernorate.

Flow cytometry analysis:

Bone marrow aspirate (BMA) samples anti-coagulated with sodium heparin were used, clotted samples were rejected. Samples were processed within twenty –four hours of extraction. Samples were prepared using gentle processing techniques. Briefly, Antibodies for CML panel were prepared using a four color panel ,which are listed as FITC/PE/PERCP/APC for each tube as follows : 38/33/45/71, 20/10/45/19, 3/16+56/45/19, 45/22/123/HLA DR, 15/117/45/34, 25/4/45/11b. The MoAB were used in different combination of flurochrome as fluorescein isothiocyanate (FITC), phycoerythrin (PE), Peridinin Chlorophyll protein complex (per cp), APC (Allophycocyanin). All the antibodies used to analyse basophils were obtained from BD biosciences. Different combination of MoAb used are, CD38(HB7), CD3 (SK7), CD20 (L27), CD15 (MMA), CD25 (2A3), CD45(2D1) were conjugated with FITC, CD33(P67.6),

CD10(MEM-78), CD16 (B73.1), CD56 (NCAM 16.2), CD22 (S-HCL-1), CD117 (104 D2), CD4 (SK3) were conjugated with PE, CD71(L01.1), CD19 (SJ25C1), CD34 (8G12), CD11B (D12), HLA DR (L243) were conjugated with APC , CD123 (7G3) conjugated with PER CP CY5.5 and CD45 (2D1) PER CP was used as a gating marker in all the tubes. The amounts of antibodies added were according to the laboratory titration.

1×10^6 White Blood cells were added to each tube and incubated at room temperature (RT) in a dark area for 15-20mins. Red cells were lysed using 1x RBC lysing reagent (Becton Dickinson USA)followed by washing cells with cell wash buffer PH 7.0-7.4 (BD) with 1%BSA(bovine serum albumin) as a blocking agent to minimize non-specific binding. In certain cases when there is non specific binding, cells were incubated for 10 minutes with 50ul of human IgG (immunoglobulin G) for blocking Fc-receptors on the cells. Finally, cells were re-suspended in a cell wash buffer with 1%bovine serum albumin(BSA) for acquisition in flow cytometer..

Acquisition was done after machine calibration by CST beads and optimization of the machine setting. Acquisition of cells was performed on BD FACS Canto2, and analysis was performed using FACS Diva V6 software (Becton Dickinson USA). The cells were gated based onCD45, side scatter allocation of abnormal cells and the expression of markers.

RESULTS:

We examined forty newly diagnosed CML cases 18 female and twenty two males age range from (22-76 years old) 38 diagnosed at the chronic phase and two cases diagnosed in the accelerated phase, also 20 control samples from regenerating normal marrow, originally diagnosed as precursor B lymphoblastic leukemia 12 pediatric patient (less than 16 y) and 8 adult patient (more than 16 years old). We found that certain markers as

CD33, CD38. CD22, CD123, CD11b are invariably expressed for every basophil benign or Malignant (Figure 1, 2). HLA-DR was variably expressed on basophil from CML and control cases in the proportion 60% and 50% respectively. CD117 was not detected on normal basophils as well as CD34 and CD25 on normal basophils while CML in accelerated phase show positivity for CD117 (Figure 3). CD11c were found on normal basophil and abnormal basophils (dim expression).

Seven cases of the control Marrow detected hematogones at different proportion (1-9%) with the characteristic pattern of hematogones. Spectrum of maturation located from the negative CD45 to the brightest location of normal mature B lymphocytes with their side scatter were lower than basophils. CD123 expression was dimmer on hematogones in the seven cases (100%) than on the basophil , while CD22 versus HLA-Dr was overlapping (Figure 4).

Table.1 show the pattern of expression of different markers on basophils in cases and control.

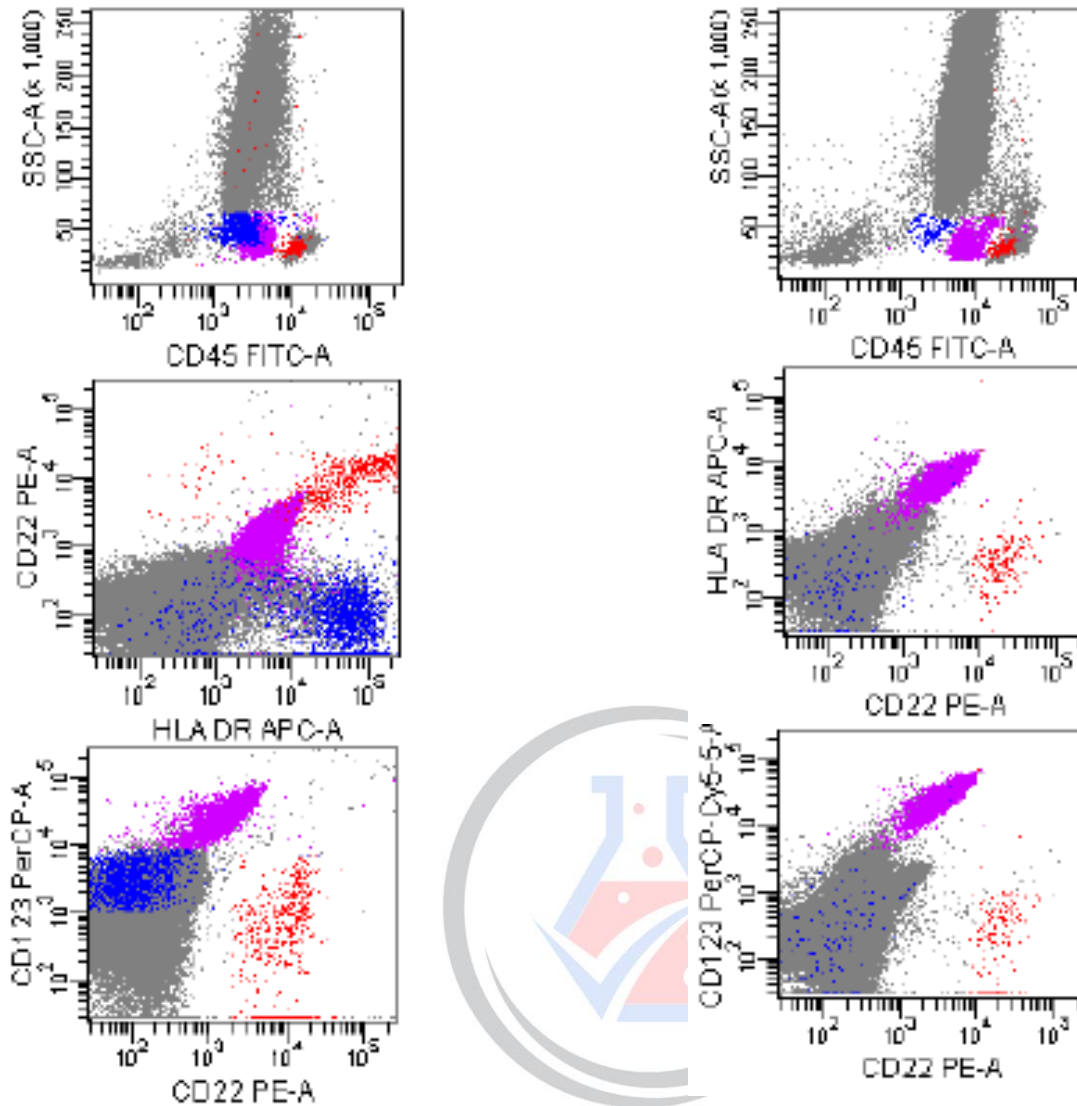| Marker	CML number (40)	Control samples number (20)
CD33	Mod	Mod
CD38	bright	Bright
CD123	bright	bright
CD22	Dim	Dim
CD11b	Dim	Dim
HLA-DR	Dim 24(60%)	Dim 10(50%)
CD117	Positive in accelerated phase (2/2) 100%	Negative
CD25	Negative	Negative

Figure. 1 show a case of CML (chronic phase) with increased basophil (Magenta). The B cell appear red in color and the blue population is myeloblast. The different expression pattern of CD123, CD22 and HLA-DR is demonstrated with bright CD123 and dim CD22 on basophils , while myeloblast are negative for CD22 while dim for CD123.

Figure. 2 show a chronic phase of CML with basophilia (magenta color). The B cell appears red in color and the blue population is myeloblast. CD123 is brightly expressed on basophil while CD22 is dimly expressed.
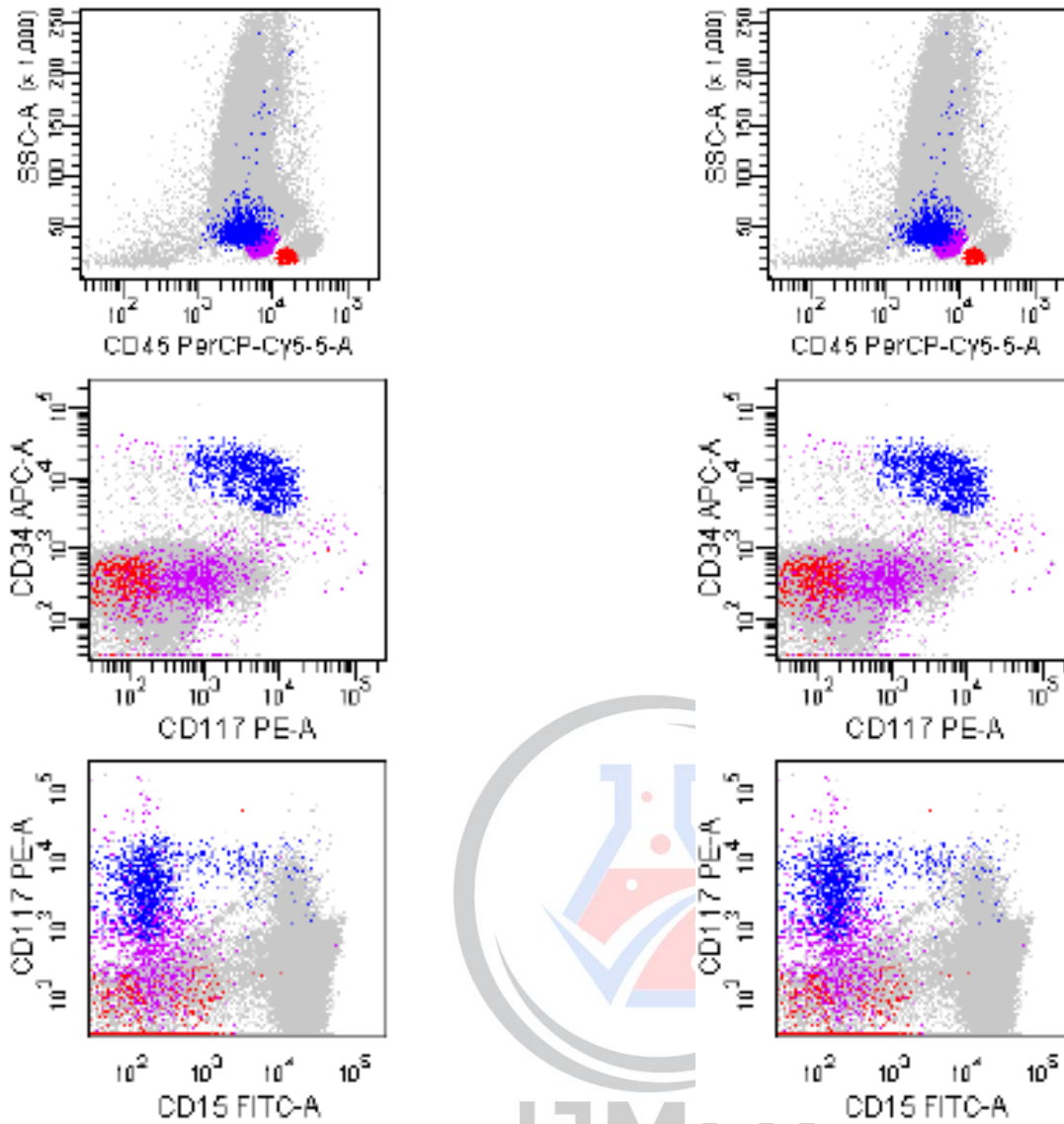
Figure. 3 show one CML (accelerated phase) case with positive expression of CD117 on basophil while the blast (blue population) positive for both CD34 and CD117 mean while B cell(red population negative for both.

Figure. 4 A control case (regenerating marrow) show the phenotypic difference between hematogones and Basophil regard CD123, CD22, HLA-DR. Basophils are Magenta in color while hematogones are red.
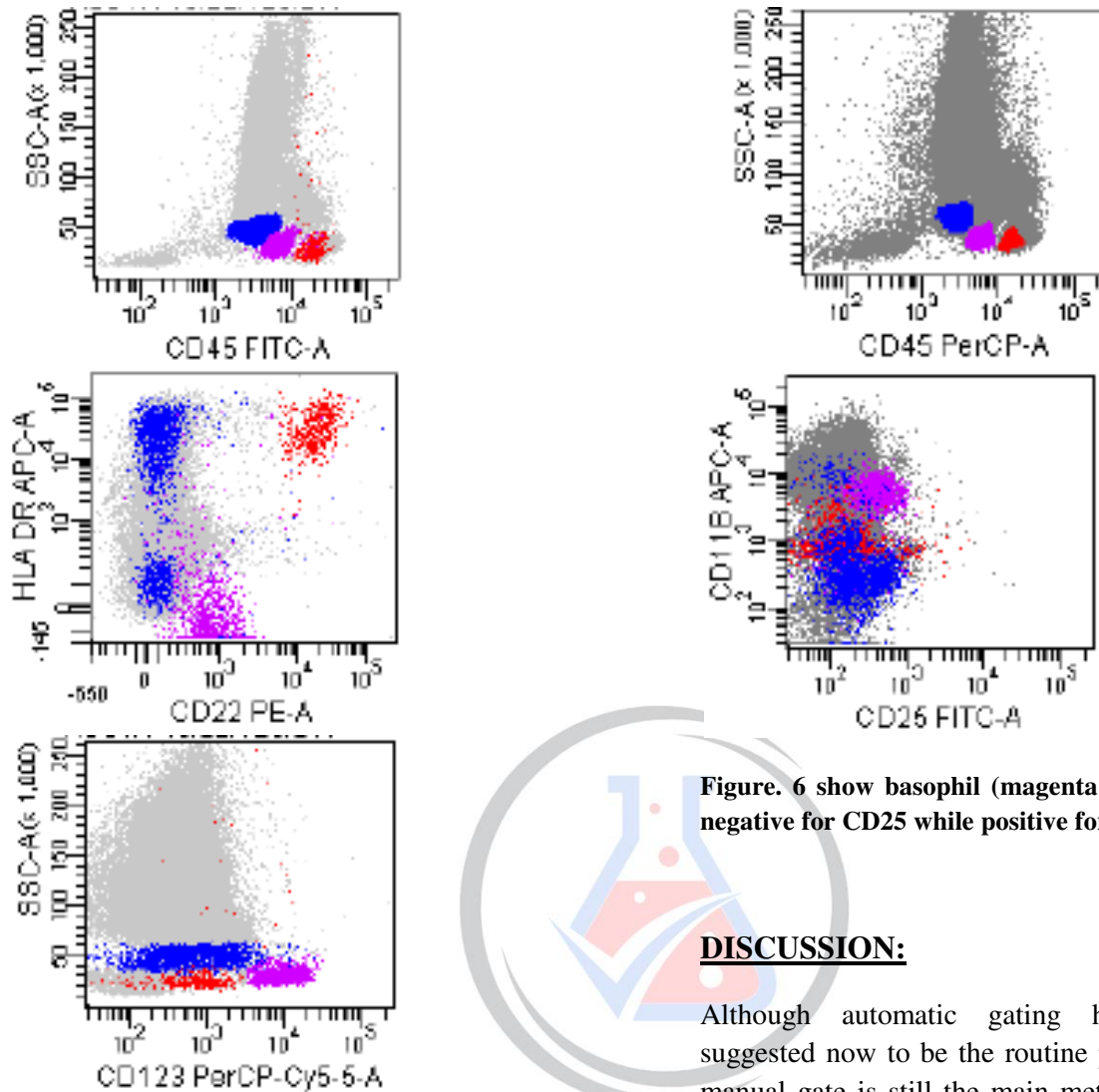
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Figure. 6 show basophil (magenta color population negative for CD25 while positive for CD11b.

Figure. 5 One CML case show an example of basophil (magenta)negative for HLA-DR while positive for CD123 (brightly) and for CD22 dimly. The B cell appears in red and carry HLA-DR and CD22 brightly, while dim for CD123.

DISCUSSION:

Although automatic gating has now been suggested now to be the routine practice, still the manual gate is still the main method for analysis especially in the low budget countries. CD45, side scatter is the standard back gating scatter gram for hematological malignancy's immunophenotyping. The area of interest is where blast could locate, it is the window area or the whole area, and normally it is occupied by normal myeloblasts and basophils mainly. In the pathological condition, it could be occupied by myeloblasts, lymphoblasts or lymphoma cells as Burkett's lymphoma. Accurate determination of occupying population is mandatory for accurate conclusion. Also in MRD, overlap between two populations could lead to confusion and mislabeling of populations detected[9,10]. Furthermore, the identification of basophilic precursors in cases of acute basophilic

leukemia and blast crises of CML with basophilic differentiation rely mainly on the knowledge of the robust markers usually used for discriminating this basophils [11].

Basophil is a minor population of mature myeloid cells, sharing myeloid markers as CD33, CD13, CD11b, as well as other markers as CD38, CD123 and CD22. This spectrum of markers expressed, could lead to the improper separation if no unique expression pattern of robust markers is specified. We examined different markers expressed on basophil in different combination and conclude that the combination of CD22 and CD123 will discriminate basophil and separate it accurately from different population located in the blast gate. The extent of CD123 on basophil is unique since it is the brightest marker detected on this cell. This strong expression of CD123 correlates it with the major role in the proliferation and differentiation of basophilis [12, 13].

CD22 dimmer expression will isolate it from mature B cells. Although, the argument about CD22 expression on precursor B lymphocytes or lymphoblast may express dim CD22, yet bright CD123 will clearly determine if it is B cell or basophil. In addition, hematogones has very low side scatter comparative to basophils and myeloblasts. Furthermore, lack of CD19 on the basophil which is a fundamental marker used in every lab.since the expression pattern of CD123 at some maturation stages of hematogones and in leukemic condition is far less than that of Basophil. Furthermore, relying on back-gating on CD45, side scatter will clearly discriminate mature B cells with bright CD45 expression. Hematogones present with heterogonous spectrum of maturation from negative to moderate CD45 and very low side scatter as well [5].

Basophil expresses bright CD38 as well as B cell precursor (hematogones) is positive for CD 38 at the same level. However both are less bright than plasma cells which typically locate at the

same area on CD45,side scatter and express unique pattern for CD38 [12]. Gating basophil using CD38 versus side scatter was not helpful but the combination of CD38 versus CD33 was able to separate basophils in control group as well as in CML cases [13].

The expression of CD11b was invariably detected on basophils in both normal and malignant condition which stand to discriminate it from myeloblast which is typically negative forCD11b [14].

Other markers as HLA-DR were recorded in 60% of the cases and around 50% of the gated basophil in the control bone marrow samples. The pattern of expression was dimmer than B cells, however comparable to myeloblasts. Very few cases does not express it, this finding is alarming since scientist use the CD123 positive /HLA-DR negative to gate basophil may lead to losing part of basophil. It is possible that DR expression is caused by cytokine activation or related to the disease itself because basophil is one of the cytokine sensitive cells [13, 15, 16].

CONCLUSION:

To conclude, the accurate quantification of different population located in the blast gate is a requirement for proper reporting at diagnosis and follow up of different hematological malignancies. The combination of CD22 and CD123 along with CD45 were definitely and precisely able to highlight and discriminate basophils from other type of cells occupy the blast gate. Identification of a single specific marker with stable expression on basophil is recommended to be investigated in the future to minimize the panel used.

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