AQP0 is a novel surface marker for deciphering abnormal erythropoiesis

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Research

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Abstract

Background

Hematopoiesis occurs in the bone marrow, producing whole spectrum of blood cells to maintain homeostasis. In addition to light microscopy, chromosome analysis, and polymerase chain reaction, flow cytometry is a feasible, fast, and quantitative analysis method to examine hematological diseases. However, because the lack of sufficient specific cell markers, dyserythropoiesis diseases are not easy to be identified by flow cytometry.

Methods: Bone marrow samples from C57BL/B6 mice and one healthy donor were analyzed using traditional 2-marker (CD71 and glycophorin A) flow cytometry analysis. After cell sorting, gene expression of membrane proteins in early and late erythropoiesis precursors and in non-erythroid cells were characterized using microarray analysis.

Results

Among characterized gene candidates, aquaporin 0 (AQP0) expresses as a surface protein in early and late stage erythropoiesis precursors and is not expressed on non-erythroid cells. In this present study, with assistant of AQP0 staining, we can define up to 5 stages of erythropoiesis in both mice and human bone marrow using flow cytometry. In addition, because patients with dyserythropoiesis generally displayed a reduced population of APQ0 high cells as compared to the normal subjects, analyses results also suggested that the levels of APQ0 high cells in the early erythropoiesis may serve as novel biomarker to distinguish normal versus dysregulated erythropoiesis.

Conclusions

AQP0 was successfully demonstrated as an erythroid differentiation marker. The expression levels of AQP0 are down-regulated in patients with dyserythropoiesis, implicating a critical and functional role of AQP0 in erythropoiesis. Accordingly, the levels of AQP0 high population in early erythroid precursor cells may serve as a reference parameter for diagnosing diseases associating with dyserythropoiesis.

Full Text

This preprint is available for download as a PDF.

Figures

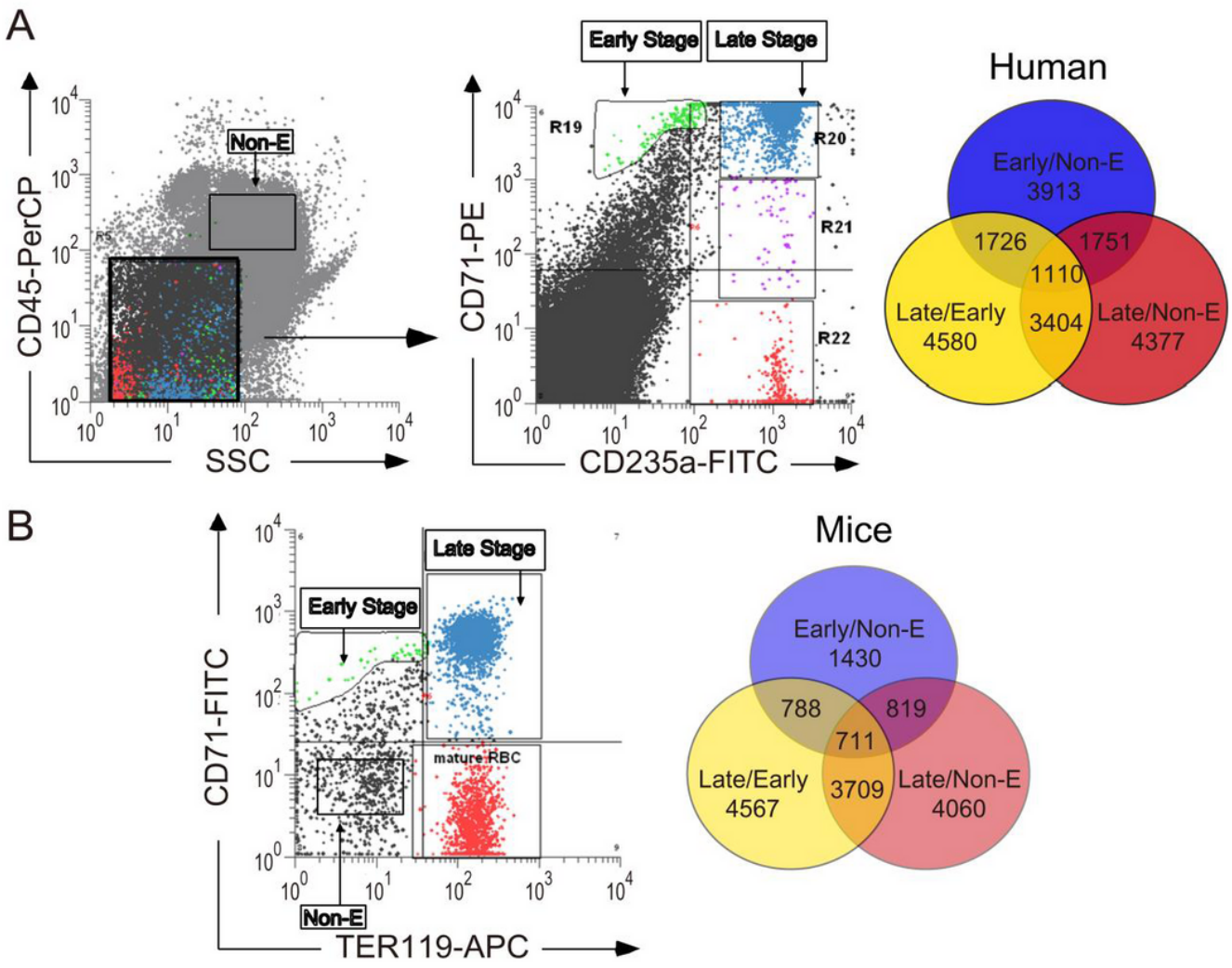

Figure 1

Idicroarray analysis of sorted erythroid differentiation stages from hum. a. mice bone nurrow. Maar, anal,is of sorted eryffrold differentiation stages from human bone nurrow (Al Human bone nurrow cells were isolated a. stained with anti-C.5 antiloches comjugated with FerCP, anti-C1371 antibodies conjugated with FE, a. ant-C.35a antibodies coml... cm, rac. A on a 0,45. caca acca-caorma ca= ams..asc. ° cells were gated ad analyzed maca ani.ara.s..— C1,71../0132353.. were coda ac nrly stage =a late stage of erythropoiesis, xespectively The numbers 3913 and 4377 .g am gene numbers specifically expressed on early and late eryimaid differentiation stages compared to non-erythroid cells>30number 4580 indica. 0e gene number specificallY expressed on late erythrold differentiation rtage compared to early erythroid differentiation stage. 'Me numbers 1751, 3404, and 1726 indicate the gene numbers shared with ea. 2-goup (earlynon-erythroid vs late/non-erybroul, latehon-ayffiroid vs latdearly, and Ike/early vs. earlyMon-erythrold). Pm number 1110 indicates Me BOO nunter shred with all 3 goups (earynon-erythroid, lateMon-erythroid, and latele.y). Idicroarray anal, of sorted erythroid differentiation stags Rom mice hone marrow (B). IVlice bone marrow cells were isolated a. gamed with ann-CD71 antibodies conjugated with Mir and conjoga. apc. acrar'd-rrnms...us wffe s.t. as non-erythroid celts..V.rnams.. .re P.rratns. were sor. as early

rage 2.1re stage of erythropoiesis, respectively The numbers 1430 and 4.0 indicate the gene numbers specifically expressed m orly and ate erythroid differentiation stages conmared to non-erythroid number 4567 indicates Me gene number specifically expr.sed 0010,¢ eryffiroid differentiation stage compared to early erythroid differentiation stage. The numbers 819 3709, and 788 indicate tlx gene numbers shared lAta each 2-group Marynon-erythroid vs. lateJnon-erythroid, latdnon-erythroid vs. We/early and latdearly vs. early,non-erythroid). The number 711 indicates the gene number shared wiffi all 3 groups (earlyMon-erythroid, lateMon-eryffiroid, 2201re/early).

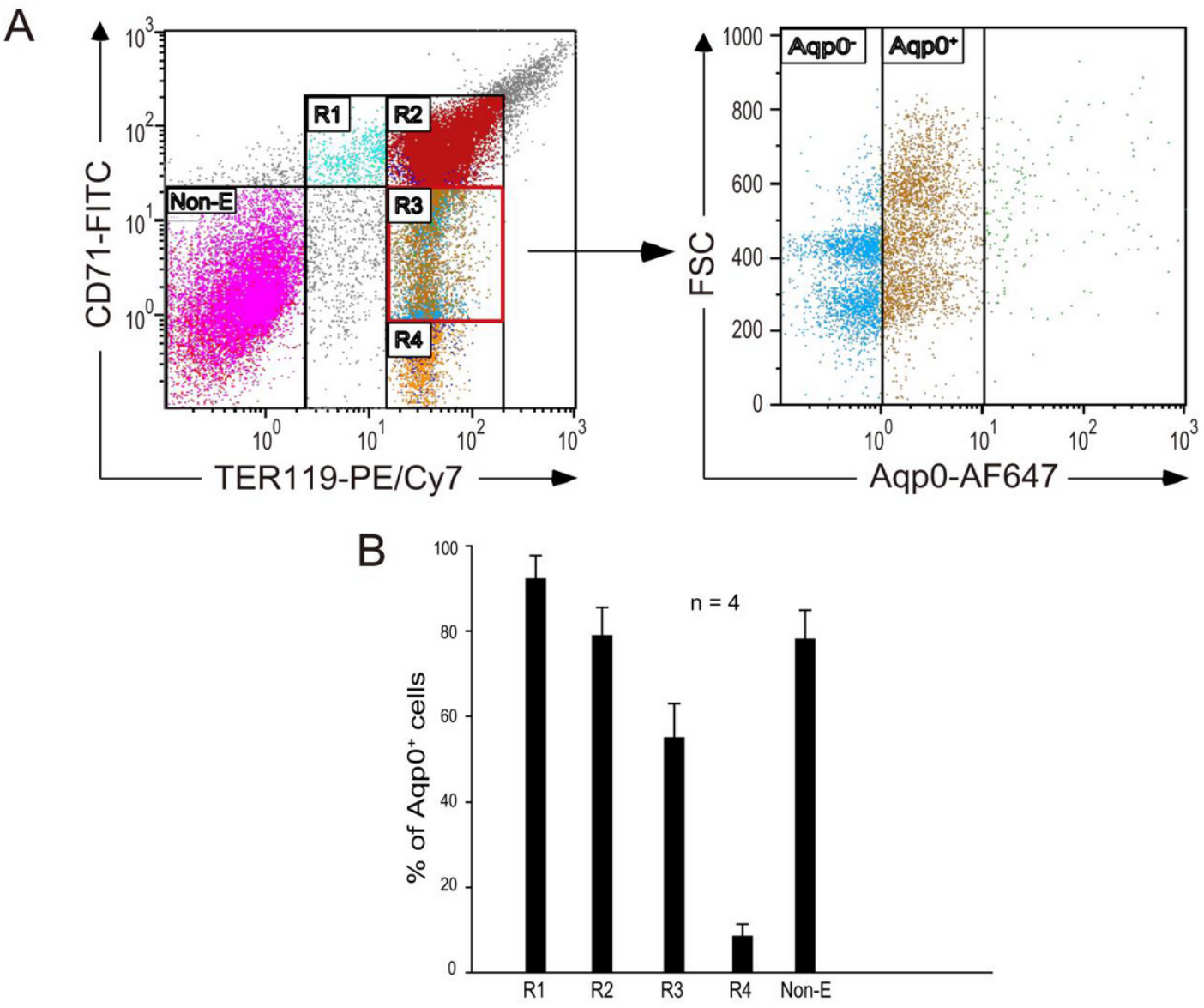

Figure 2

Charactematron f mace eryffiropmesm stages usmg 3 mrface markers (Can, .12119, and Amk) Flow crometry avalyms of mace eryffiroporems (A) Mace hone marrow cells were Isolated and slamm wed antr-C1371 antbodres conjugated me. RTC, anthodres conjugated with PriCy7, and antr-Arm0 antbodres conjugal. with Mom Fluor b1.7 C11714../FER119.° .1s were gat. as nonbywrow errs CD70.rratns.—,

can.vram00,0370-frmul9.°, and CDn-fram(.w.:reaea as region I Oil), region 2 (II2). region 3 (II3). and region 4 (M), respectively F3 was gated and analyzed the relations], between FSC (cell sere) and A))) expresmon. The percentages f Aqp0 ell m each populaton were analyzed and gum.. (B) [Mare representahve of 4 Independent expenments and reported as meal, standard demahon (SD)

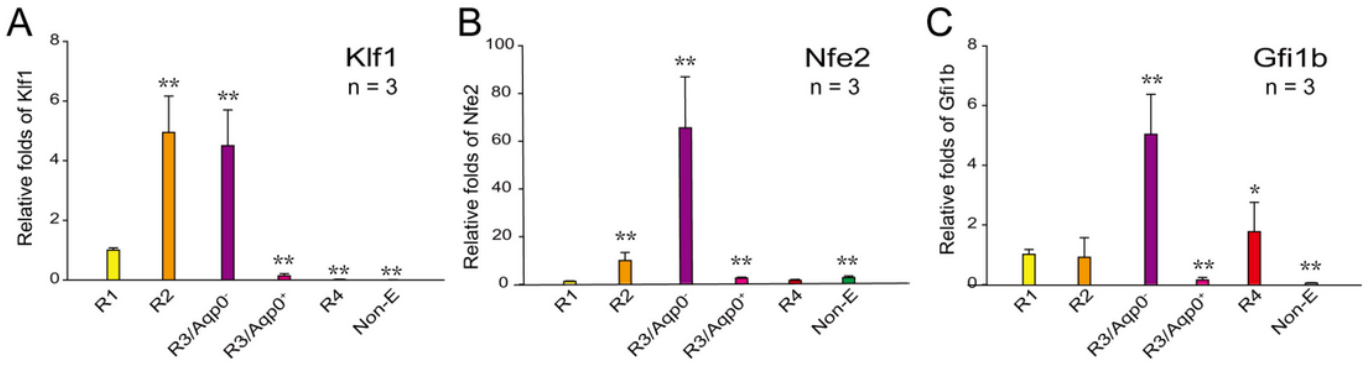

Figure 3

Relative raRNA expression of erythrold speafic transcription factors in each erythroid ...lawn stage Mace bone narrow cells were isolated and sorted as RI, RZ R3/Aqp0-, 123./..41e, R4, and Non-erythroid populations based on the fluorescence sten., a 3 erythrold speafic cell surface naarkes (CD71., TERIIO and Aqp0) The noRNA expression kvel of each gene ni RI region wss normalzed tome RIO Relative folds of IERNA expression of KM (A), 05e2 (B), and Gfilb (C) in each Cdl population conapared In RI were analyzed using quantnahve reverse polymerase ohm reachon (gRT-PGR) assay Data are representative of 3 independent experiments and reported as naean SD , < 0 05 and `, < 0 01 compared with RI groups

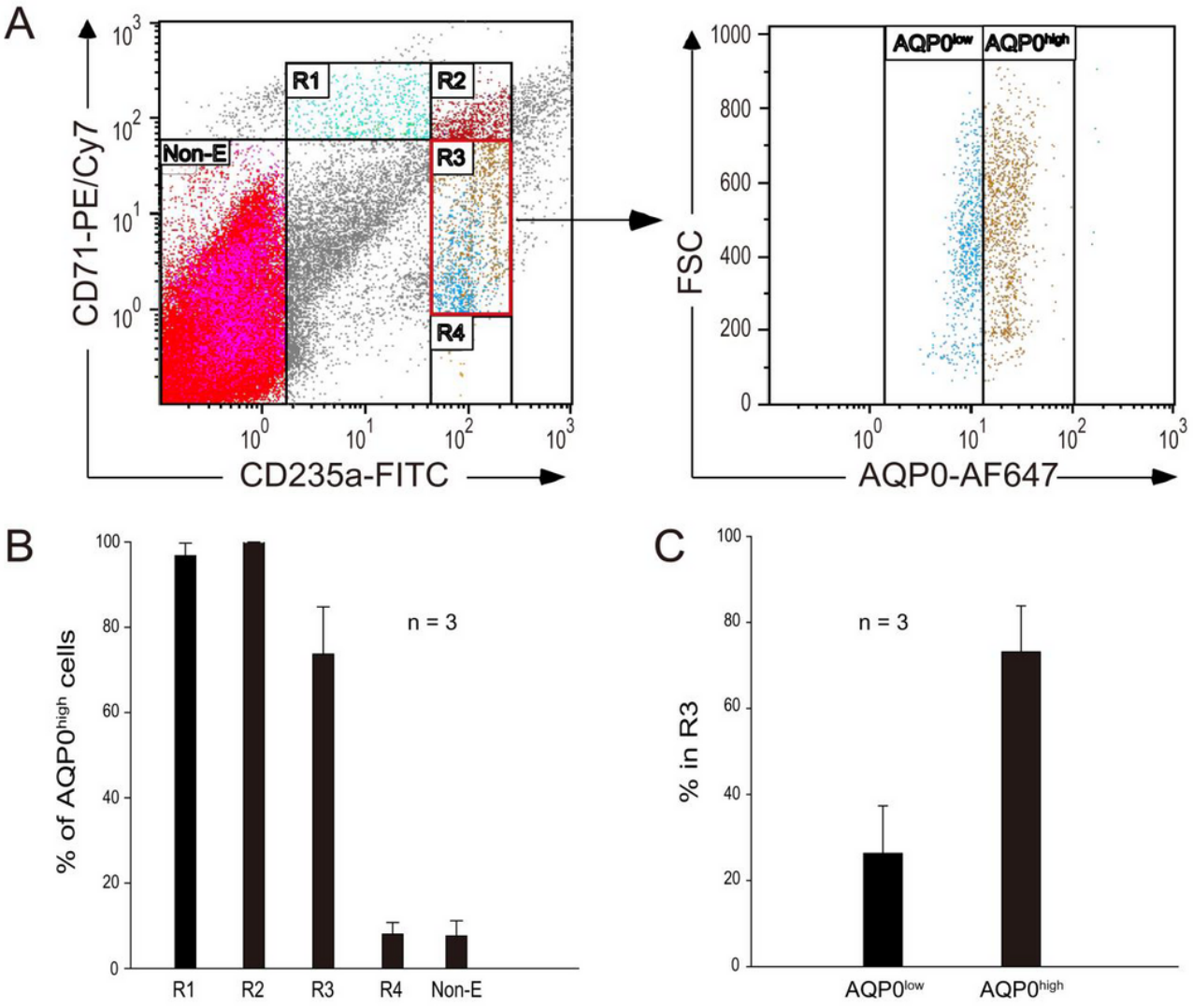

Figure 4

Charactenzahon of human erythroporems stages uslng 3 surface markers (CD71., CD235a, and AQPO). Flow cytometry analyns of human eryffiroporess (A) Hunan bone marrow cells were Isolated a. slam. with ant-CD71 antrbabes conrygated wlth. P&Cy7, arry-CD235a antbahes conplgated Coh FITC, antr-AQP0 arythodres conrygated wrth Alaa 617 CD714../C1/235.a.. cells were gated as non-erythrod cells cral../cnBsa.., CD710/C13235.a0, CD71.7.2353... crarrcre35....vffe defined as repon I (M) region 2. (02). region 3 (R3), and regron 4 OM), respectwely Cl was gated and analyzed the relatronshop between FSC (cell sue) and AQK 0presston Ihe percentages of AQP0.° cells each popul.on were analyzed a. gu.ffied (B) The percen.ges of AQP0.° and AQ40 050 002 were shown (C) D02 are representalwe of 3 Independent expenments and reported as meal, SD

A

	WBC ($10^3/\mu l$)	RBC ($10^6/\mu l$)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (%)	PLT ($10^3/\mu l$)
patient 1	6.46	4.93	14.4	41.6	84.4	29.2	34.6	260
patient 2	6.76	4.18	9.2 ↓	29.4 ↓	70.3 ↓	22.0 ↓	31.3	386
patient 3	10.01	3.07 ↓	8.7 ↓	26.2 ↓	85.3	28.3	33.2	44 ↓

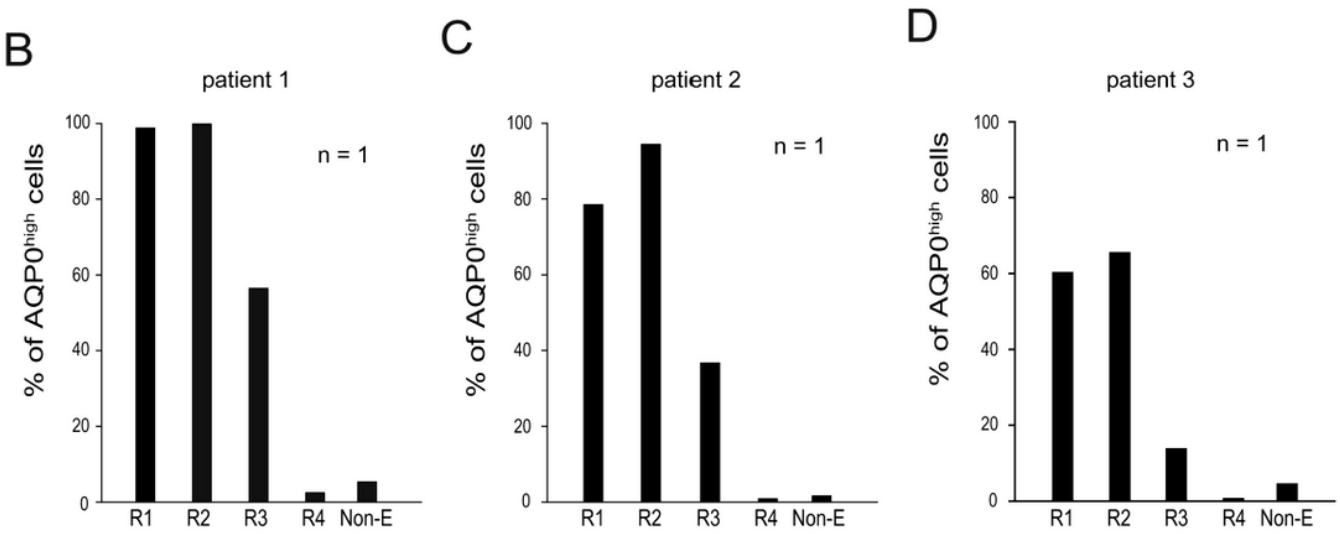

Figure 5

Charactematroa f ea-Ampex. errata. us, 3 surface markers (CD71, CO2353, and AQPO) The clmcal co.lete blood co. (CBC) values of 3 pa.. (A) lire bone marrow cells from 3 pa.. were Isolated. slam. with ant-C1371 coupgated wffla PECy7, auh-C132353 ant.d. caugated MTV, and anta-AQP0 conyugated wffla Ale. Fluor 647 C1371.-../CD235a.. cells were gat. as ...-mramo. cells C071./C132353.., CD71../.1235P., C071 `../C132353.., and C0711032353.fr were dem ®I (ID), region 2. (62), region 3 (R3), and reglon 4 (124), respecavely as was gated and analyzed we r.toosluprvrem ESC (cell sue) and AQ30 expression 113e percentages of NPO.m cells a ean populahon were amlyzed CI gum.. m patient 1 (B), pada. 2 (C), andpat. 3 (0) Data are represeutatwe of I depend. experuo.