New Insights into the Nature of the 5q- Deletion Syndrome Based on Quantitative Measurement of *BAALC*- Expressing Stem Cell Burdens – Pages 6-10

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Abstract: A discovery of nonrandom recurrent interstitial aberration at the long arm of chromosome 5 was made by Van den Berghe *et al*. in 1974. For a long time, this entity was classified as myelodysplastic syndrome (MDS). Meanwhile, its definition as well as classification criteria were repeatedly changed due to both clinical studies and advances in new techniques. In particular an insufficiency of ribosome-forming protein (RPS14) gene was found soon after similar gene *RPS19* discovery in patients with severe inherited Diamond-Blackfan anemia (DBA). It cannot be excluded that basic pathogenetic mechanisms, including participation of activated gene *TP53,* seem to be similar in both entities.

This article for the first time presents the quantitative data on the *BAALC* expression in the majority (25/31) of patients tested with 5q- deletions to be under the cut-off values. It concerns a group of 14/16 patients with isolated 5q- anomaly, and 11 other cases in whom 5q- deletion was combined with additional non-identical chromosomal aberrations. On the contrary, this molecular parameter exceeded the cut-off levels in all (n=10) MDS patients without 5q- abnormality. Hence, these data might effectively support an assumption of a ribosomopathy in cases of isolated 5q- deletion. Since about 8-10 % of these patients are transformed into MDS and/or secondary AML, a possible exclusion of isolated 5q- deletion syndrome from MDS category should be discussed carefully and this assumption is needed an additional support in larger studies.

Keywords: 5q- deletion, *BAALC*-expressing stem cells burdens, Myelodysplastic syndrome, Ribosomopathy.

INTRODUCTION

Discovery of nonrandom interstitial 5q deletion in 3 patients with macrocytic anemia resistant to standard therapy [1] and its further inclusion into category of myelodysplastic syndromes has been caused several recent problems. One of these issues was linked with further elucidation of this disorder using new technologies [2-9].

According to the first definition, the 5q- syndrome should be presented by macrocytic anemia with low-to-normal leukocyte counts and normal-to-elevated platelet counts. The bone marrow (BM) must be characterized by marked erythroid hypoplasia, hypolobulated megakaryocytes (Mg), and blast cell counts of <15% in bone marrow [1]. Meanwhile, further definitions and classification criteria have been repeatedly changed [10]. In our opinion, these controversies occured because of unusual features of isolated 5q- syndrome pathogenetically linked with haploinsufficiency of *RSP14* gene [6] and two micro-RNAs (miR-145 and miR-146a) [7], According to the present data, the development of macrocytic anemia was associated with decrease of globin synthesis coupled with heme excess [3] which correlated with activation of TP53 gene [6] closely tied with its ineffective hematopoiesis. Since myelodysplastic features develop in megakaryocytic and granulocytic lineages of hematopoiesis, they might be directly connected with abovementioned deficiencies of miR-145 and miR-146a, respectively [7]. Despite these evident molecular findings, the problem of the above syndrome classification has not elucidated yet. A novel view of this problem may be supported by our data which show lower levels of *BAALC*-expressing Stem Cells burdens in the majority of patients with isolated 5q- deletion. This finding is not characteristic for the patients with typical MDS variants. Of note, most clinical and laboratory features, as well as common pathogenetic mechanisms are similar in patients with isolated 5q- deletion syndrome and those with severe inherited Diamond-Blackfan Anemia (DBA) [9].

The present study demonstrates lower levels of *BAALC* gene expression in bone marrow cells from patients with isolated 5q- deletion syndrome supporting the discussion about its classification in the category of ribosomopathy [3, 4, 7].

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PATIENTS AND METHODS

A total of 31 patients (25 women and 6 men, aged 36 to 81 years) with an isolated 5q deletion (n=16) as well as a 5q deletion in combination with additional chromosomal abnormalities (n=15) were included in the retrospective study. As a control group, we included the data on *BAALC*-e SCs burdens measured in 10 MDS patients (41 to 81 years old) without 5q deletion. Measurements of *BAALC* expression levels which, in our experience confer to relative burdens of *BALLC*-e SCs as well as those of *WT1* were performed by means of quantitative polymerase chain reaction in real time (qPCR-RT). A cut-off values for assessment of low- and high-level *BAALC* and *WT1* expressions according our previous determinations [14-16] were equal to 31 %, and 250 copies for 10^4 copies of gene *ABL1*, respectively.

RESULTS

Basic clinical and laboratory data are presented at the Table **1**. Of importance, the levels of *BAALC* expression were under the cut-off values in 14 of 16 patients with isolated 5q- anomaly (#1-14), and eleven other patients (#17-27) in whom 5q- deletion was combined with additional non-identical chromosomal aberrations. On the contrary, this index was increased in the majority of the control group (patients #32-41). In general, 25 of 31 tested patients (80%) contained lower *BAALC*-e SCs burdens. As for other 6 patients, some of them revealed increased (over 5%) numbers of blast cells (#16, 26-31) thus indicating an advanced variant of MDS. It should be also noticed, that higher levels of *BAALC* expression (264 and 259%) were detected in two patients (#32 and #33) with normal karyotypes, followed by the patients with *EVI1*-positive AML (#34-36, 39-41) and two cases (#37 and 38) with trisomy 8.

DISCUSSION

Our study shows for the first time that 14 of 16 patients (90%) with so-called isolated 5q- deletion syndrome exhibited lower cut-off levels of *BAALC*-expressing Stem Cells. Similar results were revealed in 5 of 9 patients (56%) from the group with additional chromosome aberrations. Meanwhile, all tested MDS patients with normal or otherwise changed karyotypes had higher burdens of *BAALC-e* SCs. Moreover, the levels of *BAALC* expression in patients with DBA (n=5) were not increased too (data not shown). Since the levels of *BAALC* expression in bone marrow approximately reflect the *BAALC*-expressing Stem Cell burdens [13-15], one may conclude that this SC population in bone marrow of patients with isolated 5q- deletion could be either absent, or their small number was unable to reveal MDS [3] which will be discussed in details later. All these pathogenetic problems in patients with syndrome of isolated 5q- deletion have been discussed recently by Cazzolla [3].

Thus, many years after discovery of 5q- isolated deletion it was elucidated a mistake of its inclusion into category of MDS became evident due to its close pathogenetic ties with ribosomopathies [3, 5, 7]. Further on, clonal nature of this unusual syndrome was revealed also, what was in turn required a new explanation for development this clone in MDS. According to original Cazzoli hypothesis real pathogenesis of ribosomopathy with 5q- deletion, like that in PNH, may be dual and the role of selecting stimulator of MDS clone might belong to hypothetical gene SPARC which is known to localize also in 5q- lost region and in theory might be responsible for 5q- cells attaching to niches, However supporting evidences of this hypothethis do not fail to obtain till now. Meanwhile, our recent findings show that such a stimulator may be BAALC-e LSCs which needs to study further [4]. In our opinion. a convenient model for the insights studies of *BAALC-e* LSCs development in hematopoiesis might become the above from patients with 5q- ribosomopathy at the stage of its transformation to MDS or AML.

As mentioned above, modern pathogenesis of isolated 5q- deletion is closely connected with 5q-deletion-induced deficiencies of *RPS14*, *miR145* and miR146a genes [5-7]. *RPS14* deficiency is responsible for evolving ineffective erythropoiesis directly associated with *TP53* gene activation [5, 6]. As for miR-145 and miR-146a deficiencies, they may provide a pathogenetic basis for explanation of myelodysplastic cell features in megakaryocytic and granulocytic lineages, respectively [7].

One should be reminded that in-depth understanding of isolated 5q- deletion syndrome became possible only after advances in methodologies for the study of genes controlling synthesis of basic ribosomal proteins *RPS19* and *RPS24* which play the crucial role in pathogenesis of DBA [5], which in turn allowed in-depth studies of *RPS14* gene in pathogenesis of isolated 5q- deletion syndrome [3]. Comparative analysis shows that the basic clinical and

Table 1: Cytogenetic Findings, *BAALC* and *WT1* Expression Levels, Number of Blast Cells in Bone Marrow and Overall Survival Terms for 31 Patients with Myelodysplastic Syndromes with Emerging 5q- Anomalies

Patients, №	Age, year, gender	Diagnosis	Karyotypes	*BAALC*, % (max)	*WT1*, copies	Blasts, % (b. m.)
Isolated 5q deletion						
1	63, f	MDS	46,XX,**del(5)(q22q35)**[5]/46,XX[15]	1	2	1.2
2	59, f	MDS	46,XX,**del(5)(q13q33)[**16]/46,XX [4]	2	59	1.8
3	52, f	MDS	46,XX,**del(5)(q13q35)**[4]/46,XX [16]	3	100	1.8
4	42, f	MDS	46,XX,**del(5)(q22q35)**[7]/46,XX [8]	5	2	5
5	81, f	MDS	46,XX,**del(5)(q13q33)**[18]/46,XX [2]	6	-	-
6	78, f	MDS	46,XX,**del(5)(q13q35)**[9]/46,XX [6]	6	-	-
7	70, f	MDS	46,XX,**del(5)(q13q33)**[15]	7	-	-
8	54, f	MDS	46,XX,**del(5)(q22q35)**[19] / 46,XX[1]	8	-	-
9	56, m	MDS	46,XY,**del(5)(q13q33)**[5]/ 46,XY[5]	10	30	0.8
10	79, f	MDS	46,XX,**del(5)(q13q33)**[14] /46,XX[6]	10	-	-
11	62, m	MDS	46,XY,**del(5)(q22q35)**[15] /46,XY[5]	12	-	-
12	84, f	MDS	46,XX,**del(5)(q13q31)**[15]	13	-	-
13	64, f	MDS	46,XX,**del(5)(q13q33)**[5] /46,XX[10]	14	-	-
14	64, f	MDS	46,XX,**del(5)(q13q33)**[18] /46,XX[2]	15	-	-
15	57, f	MDS	46,XX,**del(5)(q13q33)**[15]	38	-	-
16	68, m	MDS	46,XY,**del(5)(q22q35)**[3] /46,XY[17]	62	660	5.8
Combination of 5q deletion with other chromosome abnormalities						
17	80, f	MDS	46,X,(X;16)(p11;q22)[2]/46,idem, **del(5)(q13q33)**[9]/46,XX[9]	2	-	-
18	61, m	MDS/CMPN	46,XY,del(1)(p36.1), **del(5)(q22q35)** [18]/46,XY[2]	6	-	-
19	81, f	MDS	46,XX,**del(5)(q15q33)**[18] /47,idem,+8[2]	8	-	-
20	74, f	MDS	46,XX,**del(5)(q22q35)**[13] /46,idem,del(20)(q11)[2]	12	-	-
21	77, f	MDS	46,XX,**del(5)(q13q33)[**5] /47,idem,+14[5]/46,XX[10]	23	-	-
22	66, f	MDS	46,XX,**del(5)(q13q33)**[2]/45,XX, -7,**del(5)(q13q33)**[16]/46,XX[2]	23	-	-
23	81, f	MDS	46,XX,**del(5)(q22q35)**, inv(7)(p22q11)[14]/46,XX[1]	27	-	-
24	50, m	MDS	46,XY,t(2;11)(p21;q23), **del(5)(q13q33)**	29	-	-
25	61, m	MDS - EB2	44,XY,**del(5)(q13q35)**,-13,-14[14]/ 46,XY[1]	30	234	3.6
26	46, f	MDS	46-47,X,-X,add(3)(p25), add(3)(q21), **del(5)(q13q31)**, del(15)(q22), add(16)(p11), add(18)(q23), +21, +mar[14]/ 46,XX[1]	<31	2069	8.6
27	53, f	MDS	45,XX,-7[2]/45,idem**, del(5)(q13q31)**[1] /46,XX[17]	<31	3114	9.2
28	54, f	MDS -EB2	46,XX,**del(5)(q13q33)**[3]/92,idemx2[1]/184,idemx2[1] /47,XX**,+8**[2] / 46,XX[8]	45	420	5.4
29	36, f*	MDS - EB2	ish.46,XX,t(3;15)(p22;q15),**der(5)t(5;12)(p11;q13)del(5)(q13q33),** der(8) t(1;8)(q21;p11), der(12) t(**5**;12) (??;q13)[9], der(19) t(1;19) (q21;p13), der(22)t(1;22) (q21;p12)[24XCyte]	58	111	7.4
30	63, f	MDS - EB1	52-54,XX,**del(5)(q13q33)**, **+8**,+11,+13, del(17)(p11),+19, +20, del(20)(q11)x2, +rx2,+mar[13]/104-106,idem,x2[2]	64	1384	5.6
31	68, f	MDS -EB2→ sAML	46,XX**,del(5)(q13q31)**[7]/46,idem,dup(2)(q12)[2]/47,idem,+11[3] /46,XX, t(X;**5**)(q13;**q31**)[2]/ 46,XX[1]	74	173	17.4

Selected MDS without 5q deletion						
32	48, m*	MDS- EB1	46,XY[20]{EVI1+ 16%}	264	547	5.6
33	41, f	MDS- EB2	46,XX[20]	259	2631	10
34	62, f	MDS- EB2	46,XX, **inv(3)(q21q26),** add(7)(p22), t(8;12)(q22;q13)[4] /46,XX[5]{EVI1+ 116%}	217	1441	17
35	42, m^	MDS- EB2	46,XY,**inv(3)(q21q26)**[11]/45,idem,-Y[6]/46,XY[3]{EVI1+ 69%}	154	813	12
36	81, f*	MDS - EB2	46,XX,**inv(3)(q21q26)**[8] /46,XX[12]]{EVI1+?}	119	746	18
37	56, m	MDS - EB2	48,XY,**+8**,+mar[5]	76	21	12
38	59, f	MDS - EB2	47,XX,**+8**[6]/46,XX[9]	52	n/d	16
39	52, m	MDS - EB2	45,X,-Y[8]/45,idem,i(14)(q10)[2] /46,XY[5] {EVI1+ 15%}	45	76	9
40	57, f	MDS - EB2	46,XX,**-7**,del(17)(q21),+mar[20] {EVI1+ 15%}	39	1845	?
41	44, f	MDS - EB2	46,XX,**inv(3)(q21q26)**[4]/46,XX[16] {EVI1+ 23-115%}	38 (293)	414	?

Notes: MDS-EB1 - MDS with blast excess 1; MDS-EB2 - MDS with blast excess -2; sAML – secondary AML transformed from MDS-EB2; n/d – no data: max-(maximal indicators during all course; b..m. - bone marrow; ^ treated with HSCT; *- died.

laboratory findings in patients with isolated 5q-syndrome and DBA revealed common features. The *TP53* gene activation is crucial for the both clinical entities too [4].

In the light of these findings, one may understand suggestions of some investigators to classify the isolated 5q- deletion as Ribosomopathy [4]. This viewpoint would explain the following clinical features: a) largely favorable clinical course of this syndrome; b) excellent response to lenalidomide; c) relatively rare transformation to AML; and, finally, d) lower levels of *BAALC*-e SCs burdens, what distinguishes it from other types of MDS. Of note, only a small subgroup of these patients (about 8-10%) may undergo transformed into MDS and, later, to secondary AML, which is naturally associated with increased levels of *BAALC*-e LSCs burdens.

Thus, the isolated 5q deletion syndrome, in terms of its clinical and laboratory features, supplemented by the present data on low *BAALC*, seems to be an entity different from typical MDS. However, this syndrome may be considered a ribosomopathy like DBA, with low rates (about 8-10%) of transformation to MDS and secondary AML. To test these assumptions, larger studies of patients with isolated 5q deletion are needed to obtain final evidence of this kind Stem Cells participation in pathogenesis of MDS in patients with isolated 5q- ribosomopathy.

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