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ORIGINAL ARTICLE

Red blood cell consumption in a large cohort of patients with thalassaemia: a retrospective analysis of main predictors

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Abstract

The phenotype/genotype relationship of patients with transfusion-dependent thalassaemia (TDT) is particularly complex and variable, thus generating different levels of severity and of annual transfusion volume (ATV). In this study, we explored the role and the contribution of several factors potentially involved in determining mean ATV in a cohort of TDT patients which have been followed since long time. We collected data on one-hundred and twenty-seven patients with transfusion-dependent β-thalassaemia followed at Rare Blood Cell Disease Unit, AORN Cardarelli Hospital. Age at first transfusion, genotype, spleen status (splenectomy or not), and mean soluble transferrin receptor (sTfR) were the parameters included in the analysis. At stepwise regression analysis which included all the parameters, only splenectomy and mean sTfR significantly predicted the mean ATV ($F = 70.94$, $P < 0.0001$, $R^2 = 0.540$). Overall, our data may suggest that in patients with TDT, the measurement of sTfR level together with the spleen status could contribute, more accurately than genotype, to provide a basal evaluation of residual erythropoietic activity and mean ATV.

Keywords Thalassaemia · Biomarker · Genotype · Erythropoiesis

Introduction

For long time, the standard of care for patients with transfusion-dependent thalassaemia (TDT) has been based on the lifelong administration of red blood cell transfusions in order to ameliorate anaemia and suppress ineffective and expanded erythropoiesis [1, 2]. Therefore, in these patients, iron overload occurs mainly as a consequence of transfusions. It is also becoming more and more clear that both an optimal transfusion and chelation therapy increase the survival of patients [3]. Although TDT patients are clearly different from those with non-transfusion-dependent thalassaemia (NTDT), within each group there may be great variability and some overlapping. Considering those with TDT, the majority of patients develops a severe form of anaemia (thalassaemia major) and is transfusion dependent from the first years of life depending on the severity of the disease. On the other hand, expanded erythropoiesis better characterizes patients with NTDT or thalassaemia intermedia [4], where the blood requirement is occasional or lower than that observed for TDT, but can increase as per clinical commitment. In fact, several patients with NTDT, either for the prevention or for the management of complications, are later constrained to receive regular blood transfusion (BT) and merge with TDT patients, but only for a more or less long period of their life. In fact, within the same patients, transfusion burden may be transiently different as influenced by temporary events such as pregnancy, infection or trauma allo/autoimunization, cancer, and probably, in a long-term observation, because of aging [5].

Currently, the pre-transfusion haemoglobin is homogenously and uniformly fixed by guidelines for all TDT patients, but in our clinical practice is tailored to the patients in order to obtain the best efficacy in terms of bone marrow suppression evaluated by soluble transferrin receptor (sTfR) [1, 6, 7]. In fact, the phenotype/genotype of patients

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with TDT is particularly complex and variable, thus generating different levels of severity [8]. On the other hand, transfusion burden may change from center to center, depending also on red blood cell preparation and concentration.

To our knowledge, no study has yet evaluated, with respect to genotype, the role and the contribution of several factors potentially involved in the amount of transfusion burden required in a cohort of TDT patients which have been followed in the same center since long time. Given that blood consumption fluctuates over a short-time observation, we retrospectively collected data for an observational period of 3 years.

Methods

Study population

One-hundred and twenty-seven patients with transfusion-dependent β-thalassaemia followed at Rare Blood Cell Disease Unit, AORN Cardarelli Hospital in Naples, Italy, were evaluated. All patients were previously NTDT and were started on regular BT at least from 10 years. We excluded the patients with Hb Lepore either in heterozygous or in homozygous, those with alpha globin abnormalities, all patients in pregnancy during these last years, patients that received packed red blood cells with automated washing, and the patients who were undergoing hydroxycarbamide or luspatercept therapy.

Regarding transfusion data, the volume of packed red blood cells, pre-transfusion haemoglobin (Hb) value, and the blood consumption reported in ml/kg/years, such as the age at first transfusion, the age at splenectomy, and other haematochemical parameters were retrieved. We decided to consider all these parameters recorded in our database concerning the years 2016, 2017, and 2018.

The study complied with the Declaration of Helsinki. All patients gave written informed consent to the protocol. The study was approved by the Ethics Committee of the Cardarelli-Santobono-Pausilipon Hospital, Napoli, Italy.

Genotype characterization

Blood samples were collected in EDTA and genomic DNA was extracted from peripheral blood leucocytes using the salting-out method. All coding and non-coding regions of globin gene were amplified by PCR in different fragments ranging from 200 bp to 13.4 kb and partially overlapped. α- and β-thalassaemia mutations were identified by a reverse hybridization assay (alpha and beta globin strip assay, Nuclear Laser, Vienna Lab, Austria), as already described [9].

Splenic diameter assessment

The longitudinal diameter of the spleen was measured by ultrasonography, adopting he technique of right lateral decubitus position in the coronal plane. The spleen was scanned at the left hypogastrium, it was viewed in its longitudinal axis, and the cranio-caudal length was measured from both superior and inferior poles of the spleen.

sTfR measurement

sTfR was measured with a commercially available kit using N Latex sTfR1 and BN II System (Siemens Healthcare Diagnostics) nephelometric technique.

Statistical analysis

All data were analyzed using SPSS version 18.0 statistical package. Continuous variables were described as mean ± standard deviation (SD). Categorical variables were expressed as frequencies and percentages.

The normality of distribution of the parameters was assessed by using the Kolmogorov-Smirnov test.

For continuous values with normal distribution, comparisons between groups were made by independent-samples *t* test (for 2 groups) or one-way analysis of variance (ANOVA) (for more than 2 groups). First, Levene's test was applied to verify the homogeneity of variances (homoscedasticity). When the significance level of Levene's test was < 0.05 and homoscedasticity could not be assumed, the Welch statistic was used. Wilcoxon's signed rank test and Kruskal-Wallis test were applied for continuous values with non-normal distribution. The Bonferroni adjustment was used in all pairwise comparisons.

Correlation analysis was performed using Pearson's test or Spearman's where appropriate.

Analysis of covariance (ANCOVA) models were used to evaluate the impact of potential covariates on group differences in transfusion volumes. Covariates were included if a variable was significantly different between genotypic groups and associated with the mean annual transfusion volume. In the ANCOVA, the interaction term involving the covariate was first tested for heterogeneity of slopes [10]. When homogeneity of slopes was confirmed, the subsequent model omitted the interaction and we proceeded with the standard ANCOVA model. In case of multiple covariates, the effect of each covariate was first examined separately.

Stepwise regression was performed to determine predictors of mean annual transfusion volume.

One-way repeated measures ANOVA was used to evaluate whether there was a significant difference between transfusion volumes or haemoglobin levels in the 3 years. First, Mauchly's test was used to test assumption of sphericity.

When the significance level of Mauchly's test was< 0.05 and sphericity could not be assumed, Greenhouse-Geisser corrected results were taken.

In all tests, a two-tailed probability value of 0.05 was considered statistically significant.

Results

Patient data

Table 1 shows the demographic and clinical characteristics of our patients. The 52.8% of patients were females and the mean age at the end of the study was 39.46 ± 12.71 years (range 8–75 years).

Haemoglobin, sTfR, and transfusion volumes were averaged over the last 3 years to compensate for possible annual variations.

Genotype-phenotype relationship

The phenotypic expression (β^0 or β^+) for each allele belonging to genotype was considered and three groups of patients were identified: homozygous β^+ ($N = 26$), heterozygous ($N = 59$), and homozygous β^0 ($N = 42$).

Table 1 shows the comparison among the groups. No significant differences for sex, age, and pre-transfusion haemoglobin were detected.

The homozygous β^+ patients received the first transfusion significantly later then both the heterozygous and the homozygous β^0 patients ($P = 0.030$ and $P = 0.006$, respectively).

Frequency of splenectomy was significantly higher in the homozygous β^+ group versus the homozygous β^0 group ($P = 0.030$).

The annual transfusion volume (ATV) was significantly higher in the homozygous β^0 patients than the in the heterozygous ($P = 0.012$) and the homozygous β^+ patients ($P = 0.029$) (Fig. 1).

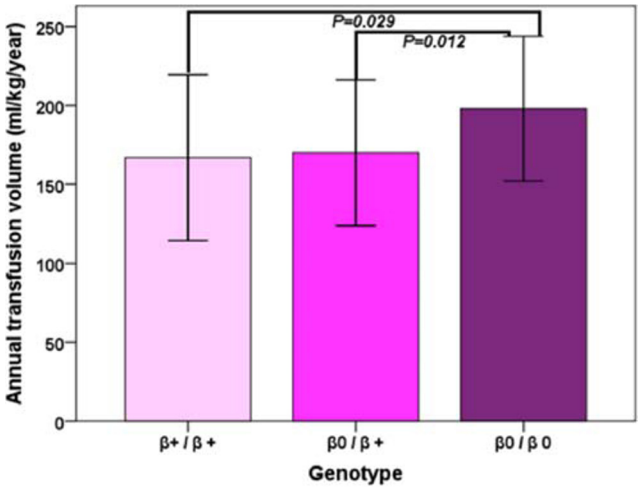

Fig. 1 Association between mean annual transfusion volume and genotype

Clinical correlates of annual transfusion volume

The ATV, considering patients overall, was higher in females, with a P value at the limits of the statistical significance (186.57 ± 45.28 ml/kg/year vs 169.67 ± 52.03 ml/kg/year; $P = 0.052$). The two sexes were comparable for frequency of splenectomy (43.3% vs 58.3%; $P = 0.090$), age (39.68 ± 11.57 years vs 39.21 ± 13.98 years; $P = 0.938$), and mean pre-transfusion haemoglobin levels (9.73 ± 0.39 g/dl vs 9.87 ± 0.38; $P = 0.051$).

A significant inverse relationship was detected between the age at first transfusion and the mean ATV ($R = -0.428$; $P < 0.0001$) (Fig. 2a). This association remained significant when only the patients who received the first transfusion before the 24 months of age were considered ($R = -0.338$; $P = 0.001$).

Table 1 Comparison among the 3 groups identified on the basis of the β-globin gene phenotypic expression

	All ($N = 127$)	β^+/β^+ ($N = 26$)	β^0/β^+ ($N = 59$)	β^0/β^0 ($N = 42$)	P
Sex (M/F)	60/67	15/11	27/32	18/24	0.469
Age at the end of study (years)	39.46 ± 12.71	39.07 ± 17.91	41.08 ± 11.61	37.41 ± 10.11	0.205
Age at first transfusion (months)^	23.36 ± 47.62 [19]	48.50 ± 97.11 [25]	20.15 ± 19.99 [23]	12.31 ± 10.59 [12]	0.007
Splenectomy, N (%)	64 (50.4)	17 (65.4)	33 (55.9)	14 (33.3)	0.019
Age at splenectomy (years)	11.07 ± 8.97	12.43 ± 11.32	10.91 ± 8.29	9.81 ± 7.63	0.737
Mean pre-transfusion haemoglobin (g/dl)*	9.79 ± 0.39	9.86 ± 0.31	9.81 ± 0.40	9.74 ± 0.41	0.421
Mean soluble transferrin receptor (mg/l)*	4.03 ± 1.71	3.79 ± 1.58	4.44 ± 1.91	3.59 ± 1.33	0.111
Mean transfusion volume (mg/kg/year)*	178.59 ± 49.13	166.82 ± 52.69	169.94 ± 46.28	198.01 ± 45.95	0.006

^ The value between square brackets represents the interquartile range (IQR)

*Mean value over the years 2016, 2017, and 2018

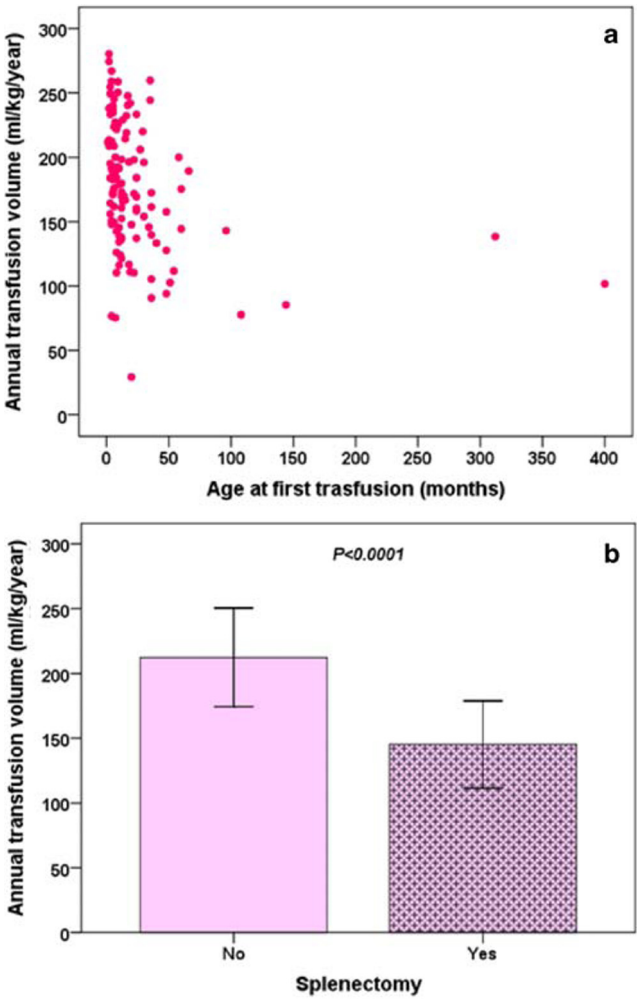

Fig. 2 Association between mean annual transfusion volume and age at first transfusion ($R = -0.428$; $P < 0.0001$) (**a**) and splenectomy status (**b**)

Splenectomized patients showed a significantly lower mean ATV than those with the spleen (145.28 ± 33.67 ml/kg/year vs 212.42 ± 38.01 ml/kg/year; $P < 0.0001$) (Fig. 2b). No significant association was detected between the age at splenectomy and the mean ATV ($R = -0.193$; $P = 0.126$). Mean duration of splenectomy was 35.07 ± 12.07 years (range 7–54 years), and this variable was not correlated with the mean ATV ($R = 0.117$; $P = 0.357$). In the non-splenectomized group, the mean diameter of the spleen was 136.14 ± 23.61 mm (range 89–190) and this variable was not correlated with the mean ATV ($R = -0.072$; $P = 0.574$).

A weak inverse relationship was detected between mean sTfR and mean ATV ($R = -0.248$; $P = 0.005$). This association was stronger when only the group of splenectomized patients was considered ($R = -0.489$; $P < 0.0001$) (Fig. 3a). Conversely, in the non-splenectomized patients, the sTfR was not significantly correlated with the mean ATV ($R = -0.206$; $P = 0.114$) but it was significantly correlated with the mean diameter of the spleen ($R = 0.315$; $P = 0.014$).

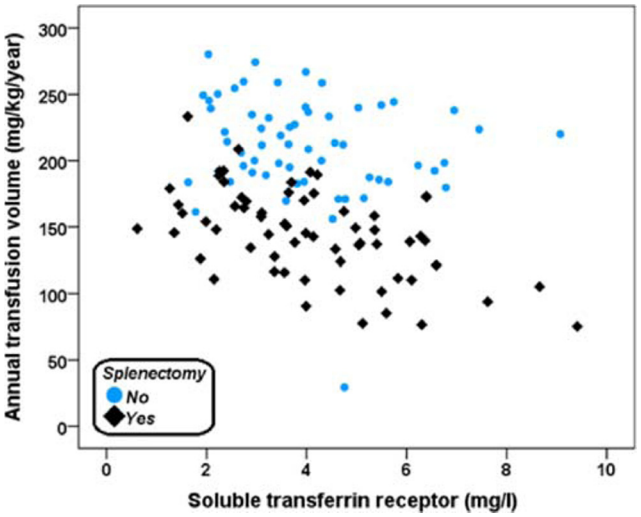

Fig. 3 Association between mean annual transfusion volume and mean sTfR ($R = -0.248$; $P = 0.005$)

Effect of covariates on the genotype-annual transfusion volume relationships

Age at first transfusion and splenectomy emerged as possible covariates and none showed an interaction with the independent variable, that is the genotypic groups ($P = 0.224$ and $P = 0.114$, respectively).

After adjustment for the effect of age at first transfusion, the association between the genotype and the transfusion volume remained statistically significant ($P = 0.021$).

After adjusting for the splenectomy status, the genotype was not a determinant of mean ATV ($P = 0.183$).

Simultaneous adjustment for both age at first transfusion and splenectomy removed the genotype-specific differences in the mean ATV (Table 2).

Predictors of annual transfusion volume

Since splenectomy was more strongly associated with the ATV than the genotype, a stepwise regression analysis was

Table 2 Results of univariate analysis of covariance for mean ATV

Variable	*F* ratio	*P*
Model 1: genotypic groups + age at first transfusion		
Genotypic groups	3.98	0.021
Age at first transfusion	10.68	0.001
Model 2: genotypic groups + splenectomy		
Genotypic groups	1.72	0.183
Splenectomy	97.11	< 0.0001
Model 3: genotypic groups + age at first transfusion + splenectomy		
Genotypic groups	1.64	0.198
Age at first transfusion	5.15	0.025
Splenectomy	87.35	< 0.0001

performed, including in the model age at first transfusion, genotype, splenectomy, and mean soluble transferrin receptor. Table 3 shows the results of multivariable analysis: splenectomy and mean sTfR statistically significantly predicted the mean ATV ($F = 70.94$, $P < 0.0001$, $R^2 = 0.540$).

Annual changes in transfusion volumes

The mean ATV in the year 2016 was significantly lower than the mean ATV in the years 2017 (170.86 ± 46.04 ml/kg/year vs 180.50 ± 50.66 ml/kg/year; $P < 0.0001$) and in respect to 2018 (170.86 ± 46.04 ml/kg/year vs 184.40 ± 54.76 ml/kg/year; $P < 0.0001$). Although this increasing trend was present for the majority of the patients, it should be noted that there were also patients who showed a decrease in the required transfusion volume with time.

The mean pre-transfusion haemoglobin in the year 2016 was significantly higher than the mean pre-transfusion haemoglobin in the years 2017 (9.86 ± 0.39 g/dl vs 9.74 ± 0.41 g/dl; $P < 0.0001$) and in respect to 2018 (9.86 ± 0.39 g/dl vs 9.78 ± 0.42 g/dl; $P = 0.004$).

Discussion

Thalassaemia syndromes are a wide spectrum of diseases ranging from red blood cell transfusion independence to transfusion dependence [11]. However, even among TDT patients, transfusion requirement is variable, depending on the severity of the disease, leading to an individual and patient-specific transfusion burden to maintain the desired haemoglobin target. Several factors have been previously recognized to determine the severity of the disease and therefore the impact on blood consumption. From a clinical point of view, it is commonly accepted that severity of thalassaemia phenotype could be linked to the age at first transfusion, but because of the remarkable genotypic diversity of thalassaemia patients and the lack of genotype-phenotype relationship, common genetic predictors of the severity of the disease are needed. On this basis, several authors recently evaluated what was the impact of known genetic modifiers on the clinical severity of thalassaemic patients with $\beta^0\beta^0$ genotype, measured by time to first transfusion. They proposed a standardized scale to better define the haematologic severity of patients to overcome the major-intermedia dichotomy, but they did not evaluate which was their influence on blood requirement [12].

Table 3 Stepwise regression for prediction of mean annual transfusion volume

	B	St. error	Beta	*t*	*P*
Intercept	242.63	8.28		29.29	< 0.0001
Splenectomy	− 66.58	6.04	− 0.68	− 11.03	< 0.0001
Mean sTfR	− 7.57	1.78	− 0.26	− 4.26	< 0.0001

As it is currently not feasible to perform an extensive genetic evaluation at our center, we wanted to explore the reciprocal relationship between the genotypic expression (β^0 or β^+) for each allele belonging to genotype and several clinical and biochemical factors encompassing the spleen status, potentially affecting transfusion burden in our patients with TDT homogenously and uniformly transfused.

Overall, our data from a quite large series of TDT patients does not confirm a pivotal and independent role of genotype and of age at first transfusion in controlling mean ATV. Conversely, the role of splenectomy and genotype seems more complex and partially linked. Splenomegaly and hypersplenism are common clinical features of TDT, but splenectomy is recommended to reduce excessive blood consumption in case of hypersplenism [13]. In our series, almost half of the patients were splenectomized and, interestingly, in the non-splenectomized ATV did not correlate with spleen diameter. However, we observed also that frequency of splenectomy was significantly higher in the homozygous β^+ group as compared with the homozygous β^0 group to the extent that, after adjusting for the splenectomy status, the genotype was not a determinant of mean annual transfusion volume. Both these observations led us to hypothesize that in the past the patients had been selected for splenectomy mainly on the basis of splenomegaly rather than of hypersplenism and that splenectomy is a marker of previous expanded erythropoiesis. Accordingly, we observed that the levels of sTfR, a biochemical maker of expanded erythropoiesis also in patients with TDT [14], was inversely correlated with mean annual transfusion and, at stepwise regression analysis, was, as well as splenectomy, predictive of it.

A beneficial role of splenectomy on parameters of iron balance was already demonstrated in a previous retrospective study from our center [15]. However, a recent Cochrane review was unable to find good-quality evidence, in the form of randomized controlled studies, regarding the efficacy of splenectomy for treating thalassaemia major or intermedia [16]. Overall, these data indicate that splenectomy and increased sTfR levels, as opposed to the $\beta^0\beta^0$ genotype, are markers of higher residual erythropoietic activity resulting in less blood consumption and probably in a more thalassaemia intermedia-like phenotype. Accordingly, we previously found that splenectomized patients, despite being regularly transfused, were more frequently affected by extramedullary haematopoiesis [14, 17]. On the other hand, it could be hypothesized that in patients with spleen, the levels of sTfR, correlated with longitudinal diameter, presumably

reflected intrasplenic marrow expansion. We have previously shown in different genotypes of NTDT that sTfR level was a good marker of erythropoiesis, independently from genotype [18, 19]. Therefore, these data may suggest that, in groups of TDT patients receiving comparable transfusion regimen, sTfR level could help to better identify, rather than the alleles composing genotype, the residual marrow erythropoietic activity, appearing as a surrogate or cumulative marker of other unknown genetic modifiers. Accordingly, in TDT patients, residual erythropoiesis marked by sTfR has been found hard to suppress even after many subsequent years of hypertransfusion and linked with the age of onset of transfusion dependence and total duration of transfusion dependence [20]. Obviously, further studies are needed for a more precise and complete evaluation of all genetic predictors of blood consumption.

The reduction of median annualized transfusion and the correction of biologic markers of dyserythropoiesis were the primary endpoints achieved during gene therapy with autologous CD34+ cells transduced with the BB305 vector [21]. This seminal study highlighted that in TDT patients, the genotype, and in particular the $\beta^0\beta^+$ condition vs that β^0/β^0, seems be predictive of a better level of residual erythropoiesis and of the discontinuation of annualized transfusion volume following gene therapy. Our data may suggest that the measurement of sTfR level together with the spleen status could contribute, more accurately than the $\beta^0\beta^0$ genotype, to provide a basal evaluation of residual erythropoietic activity in assessing candidates for this new therapy. However, further studies and larger series should be evaluated to draw definitive conclusions about this relationship.

Acknowledgments We thank all patients for their cooperation.

Compliance with ethical standards

The study complied with the Declaration of Helsinki. All patients gave written informed consent to the protocol. The study was approved by the Ethics Committee of the Cardarelli-Santobono-Pausilipon Hospital, Napoli, Italy.

Conflict of interest A.P. is the PI of the MIOT project that receives "no-profit support" from industrial sponsorships (Chiesi Farmaceutici S.p.A., ApoPharma Inc. and Bayer). The remaining authors have nothing to disclose.

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