TRANSFUSION MEDICINE

Original article

Transfusion in hemoglobinopathies and red blood cell alloimmunization: data from Sicily, Sardinia and Malta

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Background - Hemoglobinopathies are a group of diseases that include those due to globin gene mutations, such as thalassemia major (TM) and thalassemia intermedia (TI) or due to alteration of hemoglobin structure such as sickle cell disease (SCD), as well as a combination of these conditions such as thalasso-drepanocytosis (TD). They constitute the most frequent hereditary anemias requiring blood transfusion.

Materials and methods - In April 2022, a questionnaire was sent to the Transfusion Services (TS) of Sicily, Sardinia and the Maltese National Blood Transfusion (MNBT) service. The questionnaire was divided into a generic part including the number of patients followed and the type of hemoglobinopathy, and a section relating to transfusion therapy, including the number of units transfused, whether red blood cells (RBC) were washed and, finally, a section relating to the presence or absence of alloantibodies and their identification.

Results - Data was retrieved for 2,574 patients: 68.6% TM, 15.4% TI, 10.3% TD, 4.1% SCD, and 1.6% other hemoglobinopathies (OHA). The number of RBC units transfused was 76,974, equivalent to 24.5% of all the RBCU transfused from the total number of patients followed. The number of washed RBCU was 21.1% of all the units used; 337 patients (37%) were diagnosed with alloantibodies, the majority of which were patients with SCD (20.6%). Of the 485 alloantibodies found, 90.3% were identified. The antibodies found most frequently were related to the Kell system (41.7%) followed by antibodies to the Rhesus system (37.9%); 29.7% of patients had more than one antibody.

Discussion - From our study, certain indications can be formulated: 1) complete the National Registry for patients with hemoglobinopathies; 2) create a Registry of alloimmunized patients to ensure transfusion therapy is as safe as possible, considering antibody evanescence; and 3) increase the recruitment of blood donors of diverse ethnicities.

Keywords: *hemoglobinopathy, thalassemia, sickle cell disease, alloimmunization, transfusion.*

Arrived: 13 February 2023
Revision accepted: 12 April 2023
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INTRODUCTION

Thalassemia is caused by genetic defects of the globin gene which lead to anemia, chronic transfusions and co-morbidity. Transfusion-dependent patients with thalassemia major

(TM) or beta thalassemia intermedia (TI) suffer from a reduced life expectancy and from systemic pathology. This is in spite of recent improvements in clinical management that have reduced mortality and, more importantly, the current therapeutic use of gene therapy as a functional cure[1].

The most recent classification of thalassemia syndromes distinguishes autosomal recessive conditions, heterozygosity for alpha and beta thalassemia which are generally asymptomatic and do not require treatment. Homozygosity and combined heterozygosity with other thalassemia mutations determine the thalassemic syndromes. Hence the interaction between thalassemia and other hemoglobinopathies, for example, HbE, HbC, or HbS with beta thalassemia or with Hb Constant Spring (HbCS) with alpha thalassemia, will determine the presence of the various thalassemic syndromes. Currently, based on the clinical severity of these syndromes and on the necessity for chronic transfusion therapy, thalassemic syndromes can be classified into two main groups: transfusion-dependent thalassemia (TDT) or non-transfusion-dependent thalassemia (NTDT). TDT requires regular and constant transfusion therapy for survival; in the absence of this critical support, TDT patients will present with severe complications and a markedly reduced life expectancy. The TDT category includes patients with TM, while the NTDT category includes patients with TI[2].

The optimization of therapy, including iron chelation, leads to doubts as to whether there is a case for modifying this distinction, at least with regards to life expectancy[3].

Transfusion therapy remains an important support in the management of patients with SCD and TD. It is used both in acute and chronic events but may have collateral effects such as alloimmunization and iron overload[4,5].

MATERIALS AND METHODS

The collected and processed data offer a picture of the presence of hemoglobinopathies in Sicily and Sardinia, and in the Maltese islands. Similarly, as highlighted by the Italian Society of Thalassaemia and Hemoglobinopathies (SITE), not all the centers treating patients with hemoglobinopathies are identified as a transfusion service (TS). Therefore, the generic term TS is used to facilitate the description of the type of data and the activities undertaken. Clearly the management of thalassemic patients is not limited to transfusions. Following this logic, the collected data provide an assessment of the typical activities of the TS:

- number of pre-storage filtered red blood cell (RBC) units utilized, with the aim of programming collection, or the inter-regional exchange of RBC units to ensure patient access transfusion therapy;
- number of washed units, the methodology adopted and the reason for the procedure;
- whether exchange transfusions (EEX) are carried out, by which method (manual or by cell separators), and whether these processes are carried out within the TS or at other operational units.

The final part of the questionnaire concerns the type of pathology:

- which patient blood groups are defined in cases of inclusion for transfusion therapy;
- how many patients have developed irregular antibodies;
- whether these antibodies have been identified and their specificity.

Table I - *Hemoglobinopathy patients followed by transfusion services in Sicily, Sardinia and Malta identified according to the type of hemoglobinopathy*

Hemoglobinopathy	Sicily (%)	Sardinia (%)	Malta (%)	Total (%)
TM	955 (54.1)	784 (44.4)	26 (1.5)	1,765 (68.6)
TI	372 (93.0)	25 (6.3)	3 (0.8)	400 (15.4)
TD	263 (98.9)	3 (1.1)	0 (0.0)	266 (10.3)
SCD	93 (91.2)	5 (4.9)	4 (3.9)	102 (4.1)
OHA	26 (63.4)	15 (36.6)	0 (0.0)	41 (1.6)
Total	1,709 (66.7)	832 (32.0)	33 (1.3)	2,574 (100)

Percentage according to pathology in brackets. Other hemaglobinopathy/anemia (OHA) including: 12 patients with HbH disease, 1 patient with Fanconi's anemia, 6 patients with Blackfan/Diamond anemia, 1 patient with Hb Nantes, 2 patients with Pyruvate-Kinase deficiency, 14 patients with Shepherd's Bush anemia, 1 patient with HbS/Hb Lapore, 1 patient with Hb Lepore-Boston, 2 patients with spherocytosis. TM: thalassemia major; TI: thalassemia intermedia; SCD: sickle cell disease.

In April 2022, a questionnaire was sent to the TS of Sicily and Sardinia, and to the Maltese National Blood Transfusion service (MNBT) for the extraction of data relative to 2021. A total of 38 questionnaires were sent: 31 to the TS of the region of Sicily, six to the TS of Sardinia, and one to the MNBT.

The research complied with ethical requirements and all study procedures were performed in accordance with the Declaration of Helsinki.

RESULTS

The data obtained are given in the same order as requested in the questionnaire. Patients were divided according to their pathology: TM, TI, SCD, or other hemoglobinopathies/anemia (OHA).

In Sicily, 19/31 TS follow hemoglobinopathy patients, in Sardinia 6/6, and in Malta 1/1, for a total of 26 TS. Of the 26 TS which participated in the study, 2,574 patients with hemoglobinopathies were followed: 66.7% in Sicily, 32.0% in Sardinia, and 1.3% in Malta. TM was the most treated hemoglobinopathy (68.9% of all patients), followed by TI (15.5%), TD (10.3%), and SCD (4.0%), and, finally, OHA (1.6%) (**Table I**). The number of patients followed varies from a minimum of two to a maximum of 571, with a mean of 99 patients followed per TS. It should be noted that 50% of the TS followed less than 50 patients, with five TS following less than ten patients.

Data on transfusion activity

In the TS considered, during 2021, 314,333 units of RBC were transfused for all patients; 24.5% of these units (No.=76,974) were transfused to hemoglobinopathy patients with a mean number of units transfused per patient of 31.7 units. Unfortunately, it was not possible to obtain results relating to the number of units transfused for each pathology; the data relating to the units transfused are those available from the Italian National Blood Centre (CNS) in the 2022 national self-sufficiency program[6].

Units transfused to hemoglobinopathy patients were washed in 15 TS out of 19 in Sicily and 4 out of 6 in Sardinia, while units were not washed in Malta, for a total of 19 TS out of 26. The reasons for this treatment were: IgA deficiency (3/15 in Sicily and 1/4 in Sardinia; total 4/19 TS), for prevention of allergic reactions to plasma proteins (12/15 in Sicily, 3/4 in Sardinia; total 15/19 TS), to prevent post-transfusion febrile reactions (7/15 in Sicily, 3/4 in Sardinia; total 10/19), to prevent post-transfusion purpura (1/15 in Sicily, none in Sardinia; total 1/19 ST). Washing was performed manually in 14/15 in Sicily and 4/6 in Sardinia. Out of these 19 TS, one performed washing using automated techniques; the total number of washed units is 16,246/76,974 (21.1%).

Thirteen TS out of 26 (50%) follow patients with SCD, and all follow RBC exchange procedures: 8/13 using cell separators, 3/13 only manual procedures, 2/13 both manual and automated procedures. In 7/13 cases, the RBC exchange procedures are performed within the TS, while in the remaining six cases these are performed in dedicated centers specific for the management and treatment of hemoglobinopathies.

Presence of alloantibodies

Three hundred and thirty-seven patients out of a total of 2,574 patients evaluated (13.1%) developed antibodies; see **Table II** for details.

Table II - *Patients who developed antibodies*

Hemoglobinopathy	Sicily (%)		Sardinia (%)		Malta (%)		Total (%)	
	Total	Pts with allo-antibodies	Total	Pts with allo-antibodies	Total	Pts with allo-antibodies	Total	Pts with allo-antibodies
TM	955 (55.8)	98 (10.3)	784 (95.6)	124 (15.8)	26 (78.8)	4 (15.4)	**1,765 (68.6)**	**226 (12.8)**
TI	372 (21.7)	38 (10.2)	25 (3.0)	4 (16.0)	3 (9.1)	0 (0.0)	**400 (15.5)**	**42 (10.5)**
TD	263 (15.4)	41 (15.6)	3 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	**266 (10.3)**	**41 (15.4)**
SCD	93 (5.4)	18 (19.4)	5 (0.6)	2 (40.0)	4 (12.1)	1 (25.0)	**102 (4.0)**	**21 (20.6)**
OHA	26 (1.5)	4 (9.8)	15 (1.8)	2 (13.3)	0 (0.0)	0 (0.0)	**41 (1.6)**	**6 (14.6)**
Total	1,710 (100)	199 (11.6)	820 (100)	132 (16.1)	33 (100)	5 (15,2)	**2,574 (100)**	**336 (13.1)**

Subdivided by type of hemoglobinopathy and origin. Percentage of positives in brackets. TD+SCD=368 patients out of whom 62 allo-immunized (16.8%). TM: thalassemia major; TI: thalassemia intermedia; SCD: sickle cell disease; OHA: other hemoglobinopathies/anemia; Pts: patients.

Table III - *Patients with alloantibodies, subdivided by pathology and sex, average age and relative standard deviation, minimum and maximum age*

Hemoglobinopathy	Patients	M (%)	F (%)	Average age ± SD	Min/Max
TM	226	111 (49.1)	115 (50.9)	40.7±12.9	4/60
TI	42	18 (42.8)	24 (57.1)	50.8±10.2	30/75
TD	41	19 (46.3)	22 (53.6)	47.2±11.8	20/64
SCD	21	9 (42.8)	12 (57.1)	43.1±17.7	4/72
OHA	6	2 (33.3)	4 (66.7)	50.5±20.3	20/74
Total	336	159	177	43.1±13.4	4/74

SD: standard deviation; M: male; F: female; TM: thalassemia major; TI: thalassemia intermedia; SCD: sickle cell disease; OHA: other hemoglobinopathies/anemia.

Table IV - *Identified antibodies, absolute number and as a percentage of the total identified antibodies, and by pathology*

Antibodies	TM (%)	TI (%)	TD (%)	SCD (%)	OHA (%)	Total
A1	0 (0.0)	0 (0.0)	1 (1.5)	1 (2.6)	0 (0.0)	2 (0.5)
D	16 (6.0)	2 (3.4)	2 (3.1)	2 (5.7)	0 (0.0)	21 (5.0)
C	10 (3.7)	4 (6.8)	2 (3.1)	1 (2.6)	0 (0.0)	16 (3.7)
E	24 (9.0)	7 (11.9)	9 (13.8)	7 (18.4)	0 (0.0)	47 (10.7)
c	5 (1.9)	5 (8.5)	4 (6.2)	4 (10.5)	1 (11.1)	19 (4.3)
e	1 (0.4)	3 (5.1)	1 (1.5)	0 (0.0)	0 (0.0)	5 (1.1)
Cw	36 (13.4)	5 (8.5)	8 (12.3)	4 (10.5)	2 (22.2)	55 (12.5)
V	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
f	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.6)	0 (0.0)	1 (0.2)
G	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
K	40 (14.9)	8 (13.6)	8 (12.3)	2 (5.3)	0 (0.0)	58 (13.2)
k	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Kpa	98 (36.6)	9 (15.3)	12 (18.5)	5 (13.2)	1 (11.1)	125 (28.5)
Kpb	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Jsa	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Jsb	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fya	6 (2.2)	1 (1.7)	2 (3.1)	2 (5.3)	1 (11.1)	12 (2.7)
Fyb	1 (0.4)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.2)
Jka	11 (4.1)	2 (3.4)	1 (1.5)	0 (0.0)	2 (22.2)	16 (3.7)
Jkb	4 (1.5)	0 (0.0)	3 (4.6)	2 (5.3)	0 (0.0)	9 (2.1)
Lea	2 (0.7)	1 (1.7)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.7)
Leb	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
S	3 (1.1)	1 (1.7)	4 (6.2)	1 (2.6)	0 (0.0)	9 (2.1)
s	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.6)	2 (22.2)	3 (0.7)
M	1 (0.4)	2 (3.4)	3 (4.6)	2 (5.3)	0 (0.0)	8 (1.8)
N	0 (0.0)	3 (5.1)	0 (0.0)	0 (0.0)	0 (0.0)	3 (0.7)
P1	1 (0.4)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	2 (0.5)
Lua	3 (1.1)	5 (8.5)	2 (3.1)	1 (2.6)	0 (0.0)	11 (2.5)
Lub	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Coa	1 (0.4)	0 (0.0)	1 (1.5)	0 (0.0)	0 (0.0)	2 (0.5)
Cob	1 (0.4)	0 (0.0)	0 (0.0)	1 (2.6)	0 (0.0)	2 (0.5)
Ytb	0 (0.0)	0 (0.0)	1 (1.5)	1 (2.6)	0 (0.0)	2 (0.5)
HLA	4 (1.5)	1 (1.7)	0 (0.0)	0 (0.0	0 (0.0)	5 (1.1)
Total (%)	267 (100)	59 (100)	65 (100)	38 (100)	9 (100)	438(100)

TM: thalassemia major; TI: thalassemia intermedia; SCD: sickle cell disease; OHA: other hemoglobinopathies/anemia.

Out of the patients in whom alloantibodies were found, 159 were male (47.3%) and 177 female (52.7%). Patients' average age was 43.1±13.4 years; the youngest was 4 years old and the oldest 75 (**Table III**).

Two out of the 336 patients with positive screening (0.6%) presented with cold antibodies (both affected by TM), 13 (3.9%) with pan agglutination (9 TM, 1 TI, 2 SCD), 29 (8.6%) with unidentified antibodies (16 TM, 4 TI, 6 TD, 2 SCD, 1 OHA), and two (0.6%) with identified and unidentified antibodies (1 TD, 1 SCD).

Four hundred and eighty-five antibodies were found, and from these 438/485 (90.3%) were identified. For the purpose of the further evaluation of the data, only the identified antibodies were considered (296 patients). **Table IV** reports the details of the identified antibodies subdivided according to pathology.

It is to be noted that antibodies relating to the Kell system represent the highest percentage of antibodies (41.7%), particularly anti Kpa (28.5%) and anti Kell (13.2%), while 37.9% of all the antibodies identified relate to the Rh system, especially anti Cw (12.5%) and anti E (10.7%). The remaining 19% of antibodies related to the other blood group systems. Anti-HLA antibodies were 1.1% (data submitted by only 1 TS). The percentage of the frequency of antibodies is reported in **Figure 1**.

In 70.3% of patients, a single antibody was identified, in 29.7% of patients, multiple antibodies were found. Thirteen patients had a mixture of 4-6 antibodies; five were male (38.5%) and eight female (61.5%), average age 41.7±21.5 years, two with TM (15.4%), two with TI (15.4%), five with TD (38.5%), three with SCD (20.1%), one with OHA (7.7%) (**Table V**).

DISCUSSION

Our survey has allowed us to obtain data relating to a large number of patients distributed across TS for a total 2,574 patients; this number was compared to data obtained from the census carried out by the SITE[7] in which the number of transfusion-dependent thalassemics (TDT) followed in Sicily and Sardinia is 2,234 patients, ie., 43% of all TDT patients followed in Italy. This slight difference in figures is probably due to the fact that our data are obtained directly from TS which perform cross-matching and which, in the majority of cases, also provide transfusion therapy for non-hemoglobinopathy patients. Such patients still require transfusion therapy (e.g., OHA patients). In contrast, the SITE generally targets centers which follow hemoglobinopatic patients, including NTDT. Transfusion therapy heavily affects patient management, particularly in the regions of Sicily and Sardinia which

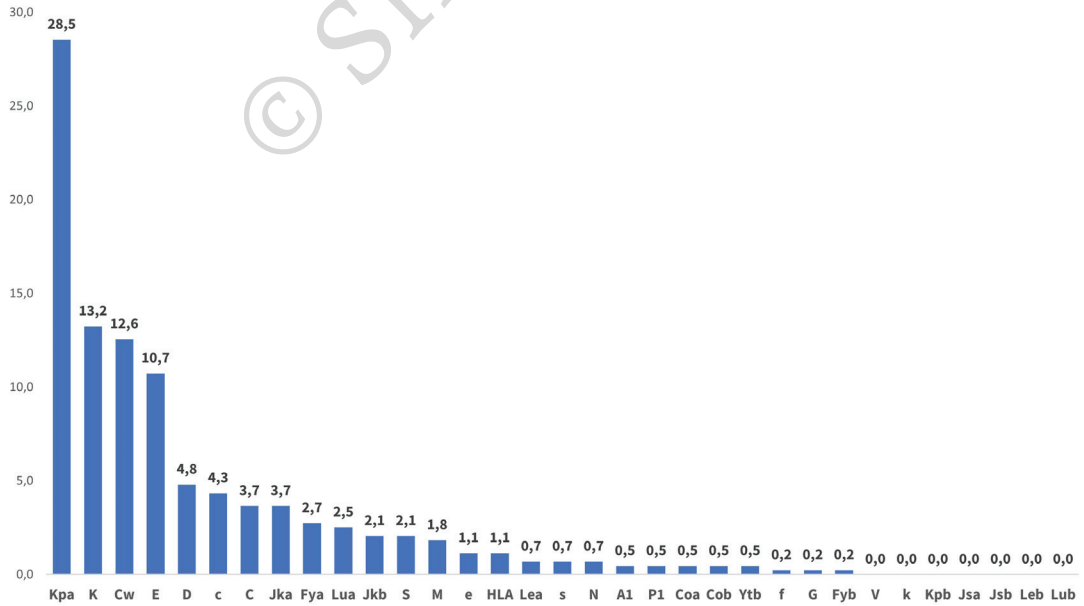

Figure 1 - Percentage frequency, in descending order of antibodies encountered

Table V - *Mixture of 4-6 antibodies: patients' characteristics and identification of antibodies present*

Antibodies N.	Sex	Age	Pathology	A1	E	c	Cw	f	K	Kpa	Fya	Jka	Jkb	Lea	S	s	M	P1	Lua	Coa	Cob	Ytb
4	F	34	TI				Cw			Kpa				Lea					Lua			
4	M	62	TD		E		Cw				Fya						M					
4	F	56	AIHA			c	Cw				Fya					s						
4	F	62	D		E						Fya		Jkb		S							
4	F	29	D		E	c	Cw			Kpa												
6	F	42	TD			c					Fya				S		M		Lua	Coa		
5	F	57	TD	A1	E		Cw		K	Kpa												
6	F	63	D				Cw	f	K	Kpa											Cob	Ytb
6	F	60	TD						K	Kpa			Jkb		S		M	P1				
4	M	35	TI		E				K	Kpa									Lua			
4	M	31	TD		E	c			K	Kpa												
4	M	5	TM				Cw			Kpa	Fya		Jkb									
4	M	6	TM				Cw			Kpa	Fya	Jka										

TM: thalassemia major; TI: thalassemia intermedia; TD: thalasso-drepanocytosis; D: drepanocytosis; AIHA: autoimmune hemolytic anemia.

have the highest number of patients in Italy and where the RBC units transfused to hemoglobinopathy patients represent, respectively, 25% and approximately 33% of all the RBC units transfused to all patients, with an economic value, according to current rates, of over 14 million euro: 8.4 million euro for the region of Sicily and 6.1 million euro for the region of Sardinia[8].

RBC washing involves 73% of all the centers participating in the study, and both the rationale for which this is performed and the procedure adopted vary greatly. The main reason for which the procedure was performed was to prevent allergic reactions to plasma proteins, performed in 15 centers out of 19 (79%), while automated washing, considered the optimum method, is performed in only one center. This leads us to believe that the various indications in the guidelines (documented IgA deficiency, recurrent allergic reactions not responsive to antihistamines, febrile post-transfusion reactions, renal insufficiency) are only partially followed and there may be other reasons which the participating centers did not declare.

It should be noted that RBC washing, which in the literature is considered critical for 10-15% of patients, leads to a significant use of resources, and up to a 20% reduction in hemoglobin and a reduction in RBC quality. In fact, if an open system is used, there is a risk of contamination of the units[5,9].

RBC exchange transfusions are performed in 13 of the 14 TS which manage these patients, and of these 8/13 use cell separators, 3/13 manual procedures, and 2/13 both automated and manual procedures, depending on patients' characteristics. According to the evidence provided in the latest update of the American Society for Apheresis (ASFA)[10], automated RBC exchange is more efficient in removing/replacing deformed cells than manual methods or simple transfusion. Exchange transfusion may also have a beneficial effect on blood viscosity, blood vessel elasticity, the reduction in adhesion molecules, and cerebral blood supply. Iron overload may be treated using iron chelating agents or therapeutic phlebotomy. RBC exchange, especially with the resulting isovolemic hemodilution, may remove or reduce iron deposits, even if some authors, in recent meta-analysis, do not agree with such conclusions[11].

Extended phenotypic and genotypic determination of RBCs before commencing transfusion therapy include C, c, D, E, e, Jka, Jkb, Fya, Fyb, Kpa, Kpb, MNS, Lewis; however, although recommended by various guidelines[5-12], this is generally not followed.

Alloimmunization

The development of alloantibodies against RBCs is well known and is a frequent complication of chronic transfusion therapy. Various TS throughout the world have reported a varying frequency of alloimmunization. Hemoglobinopathy patients are transfused with RBC concentrates typed for ABO and Rh D. On the other hand, more recent authors recognize the importance of counting the number of units transfused and the incidence of the first alloimmunization[13].

In our study, irregular antibodies were found in 336 of the 2,563 patients (13.1%). For the aim of our survey, 40 patients with unidentified antibodies were excluded. Pan reactivity in screening for antibodies was one of the most complicated problems to solve when identifying units for transfusion. This may be due to cold autoantibodies which require adsorption techniques to exclude the potential presence of alloantibodies. When the direct antiglobulin test (DAT) is negative, the possibility that the reaction is due to an antibody against a high frequency antigen or a mixture of specificities must be considered and excluded. Furthermore, some pan reactive antibodies with the indirect antiglobulin test (IAT) may be linked to the technique or reagents used. Unfortunately, our survey did not include information relating to the techniques used by the various TS for the identification of antibodies[14]. Hence only the 438 antibodies identified in 296 patients were assessed. For details on the distribution of the various antibodies in the respective hemoglobinopathy, see **Table III**.

In our survey, the average number of RBC units transfused for all patients, regardless of the type of hemoglobinopathy, was 31.7 units/year with an assumed inter-transfusion interval of 11.5 days. The average age of alloimmunized patients, which in our case is 43.1±13.4 years, is higher than that encountered in other studies[15], in which the average age varied from a minimum of 6.7 years to a maximum of 26.3 years for TM and 36.9 for TI. The higher average age of alloimmunized patients with respect to other studies suggests that our patients have lived through all the progress that transfusion medicine has made in the last 30-40 years. During this time, units of blood have been collected in glass bottles and plastic materials, manual and automated whole blood separation techniques have been adopted, blood units have been unfiltered or filtered at the bedside or undergone pre-storage filtration with transfusion criteria which considered the pre-transfusion hemoglobin level; many of these procedures are no longer used. The evolution of laboratory methods in compatibility testing and the identification of alloantibodies should also be considered.

In our study, the percentage of patients who presented with antibodies was 13.1% (11.6% in Sicily, 16.1% in Sardinia, 15.2% in Malta) (**Table II**). In other studies[15,16], the percentage of alloimmunization varies from a minimum of 2.9% to a maximum of 37%[15], and, in another study, from a minimum of 1.82% to a maximum of 18.75%[18]; all these studies show heterogeneity in the data.

In a recent article relating to patients with SCD and TD[17], the percentage of alloimmunized patients is 8.5% *versus* the 16.8% identified in our study.

As regards the antigens against which our patients developed antibodies, 41.7% of antibodies encountered involved the Kell system (41.7% of all antibodies) with 28.5% of the antibodies directed against Kpa. Some authors[6], including British authors[18], do not consider antibodies against this antigen to be clinically significant. Others[19] and case reports describe these antibodies as being clinically significant since slight/moderate delayed hemolytic reactions both in adults and children were described[20-24].

Processed data obtained from the Rare Blood Group Bank of Ragusa, Sicily, show that out of 14,830 donors tested, 278 (1.8%) resulted positive for Kpa (*Giuca G and Travali S, personal communication, 2023*); these data are in line with those in the literature[21]. Hence the presence of such a high number of anti Kpa antibodies contrasts with reports by various authors of a high variability in the data, with values that vary from 4 to 20% of the antibodies encountered. The average age of patients also varies from 9 to 14 years[25], although such information is not always provided[26]. The wide diversity in the various reports could also be related to the overall number of RBC units transfused[27]. Whether an extended phenotype/genotype of patients and the number of units transfused specifically in hemoglobinopathies are available should also be taken into consideration[28].

Our survey was directed at a patient population which represents a wide age range. For many years, transfusion therapy in Italy has required matching for Rh phenotype

for multi-transfused hemoglobinopathy patients. In this way, the presence of antibodies directed against the Rh system is reduced over time, while the availability of ever more sensitive immune-hematologic techniques which can identify minor antigens has increased. One must also consider that there may be erythrocyte panels with cells lacking certain antigens, for example, Kpa antigen, which, given the reduced expression in Caucasians, may lead to the presence of specific antibodies being underestimated. Extended typing of both patients and donors shows evidence of a high prevalence of alloimmunization linked to various blood groups[29]. Pre-existing antibodies due to evanescence, or a history of such information, may also be missing. All these factors could lead to a delay in transfusion reactions[30].

For some authors, assessment of problems related to logistics and cost are also important considerations[31], as are the need to obtain the consent of patients and donors for extended typing, the dissemination of such data, and the management of donor privacy[32].

In our data, a mixture of antibodies (2-6 different antibodies) was detected in 29.7% of patients, with 13 patients presenting a mixture of between four and six antibodies (**Table V**). In these cases, the search for compatible blood units may be particularly complicated and financially demanding.

Pregnancy represents an event that may determine the appearance of alloantibodies. However, the possibility of pregnancy is markedly reduced in thalassemic females since hypogonadism is particularly present in these patients. Therefore, very few studies have shown evidence of immunogenicity linked to a possible pregnancy.

The situation is different for females with SCD, who may have normal pregnancies. Here the guidelines of the American Society of Hematology (ASH) recommend matching for Rh antigens (C, E, o, C/c, E/e) and K besides ABO/RhD for SCD (all genotypes) patients in case of transfusion. The extended RBC antigenic profile may be genotypically or phenotypically determined, and extended RBC antigenic matching (Jka/Jkb, Fya/Fyb, S/s) may also provide further protection from alloimmunization[4].

CONCLUSIONS

The results of our survey lead us to formulate the following indications.

Fully establish the National Registry for Hemoglobinopathy Patients, as set out in the legislation[33].

Insofar as the data available is concerned[34-36], the current state of hemoglobinopathy patients in Italy and Malta does not seem to be adequately represented, compromising the comparison of data[37].

Create a national network of Hemoglobinopathies Centers to take over all patient management with the operational ability to provide all the services necessary to ensure the optimal take up of patients, in order to:

- identify diagnostic-therapeutic pathways and assistance which are uniform and coherent according to appropriate criteria, effectiveness and efficiency;
- simplify the access to Hemoglobinopathies Centers through the implementation of a direct access channel and the development of diagnostic and therapeutic pathways;
- ensure a high level of standardized treatment;
- overcome territorial differences in health service provision, ensuring that services are available throughout the area to facilitate patient access;
- disseminate research results and favor access to innovative therapies;
- promote the prevention of clinical risk and the diffusion of good practice, to guarantee quality and safety of care[38]. In this context, it would be useful to standardize the immune-hematologic status should transfusion be required since not all TS perform an extended typing for RBC antigens, as already required by international guidelines[4-39];
- set up within the Italian Registry of Rare Diseases a section in which to register alloimmunized subjects with hemoglobinopathies to increase transfusion safety, considering the evanescence of antibodies[40,41];
- facilitate the search for compatible units, even if the patient has to be transfused outside the referral center, or at least provide all alloimmunized patients with a document showing their blood group phenotype, especially information about possible development of antibodies, explaining to the patient how this information should be used[42,43];
- increase blood donor recruitment of diverse ethnicities considering the increased presence of subjects and patients coming from various nations many of whom have also overcome social and legal difficulties, and

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favoring social inclusion in safeguarding the security of the patient and the donor[17, 44,45];

- given that there is an ever-increasing number of children born of mothers with non-Italian nationality (in 2021, 9.9% of deliveries were from mothers with non-Italian citizenship), such phenomenon is more diffuse in areas of the country with the highest foreign presence, namely central and northern Italy where over 30% of births are traced to foreign mothers. The geographical areas most represented are Africa (28.0%) and the European Union (21.4%), while mothers originating from Asia and South America constitute respectively 19% and 18.7% of foreign mothers[46].

Our retrospective study shows some limitations as regards missing information on the type of tests performed for antibody detection and identification. However, it would have been very complex to obtain such information as the average age of our patients covers a period of over 40 years and the technological evolution that has taken place in the field of transfusion medicine makes it impossible to track such information. Information relative to the date of the first transfusion and the finding of the first alloantibody is also missing. Neither was any distinction made between the pathologies of the RBCs transfused; we referred to a presumed average of units utilized. It is not possible to determine whether the patients have to attend a different TS than that in which the compatibility testing is performed for their check-ups and to receive therapy for any complications of the various hemoglobinopathy types.

AUTHORSHIP CONTRIBUTIONS

GG and RM designed the study and reviewed the paper. AA reviewed the paper. GG managed the data. The SIMTI Regional Section of Sicily and Sardinia and the Maltese National Blood Transfusion (MNBT) Service were responsible for data entry. All Authors and and Co-Authors approved the final version of the paper.

The Authors declare no conflicts of interest.

REFERENCES

1. Chapin J, Cohen AR, Neufeld EJ, Vichinsky E, Giardina PJ, Boudreaux J, et al. An update on the US adult thalassaemia population: a report from the CDC thalassaemia treatment centres. Br J Haematol 2022; 196: 380-389. doi: 10.1111/bjh.17920. Epub 2021 Nov 14. PMID: 34775608.
2. Cappellini MD, Farmakis D, Porter J, Taher A. Guidelines for the management of transfusion dependent thalassaemia (TDT). 4th edition (2021), Thalassaemia International Federation. Available at: https: //thalassaemia.org.cy/it/tif-publications/guidelines-for-the-management-of-transfusion-dependent-thalassaemia-4th-edition-2021-v2/. Accessed on 19/01/2023.
3. Vitrano A, Calvaruso G, Lai E, Colletta G, Quota A, Gerardi C, et al. The era of comparable life expectancy between thalassaemia major and intermedia: Is it time to revisit the major-intermedia dichotomy? Br J Haematol 2017; 176: 124-130. doi: 10.1111/bjh.14381.
4. Chou ST, Alsawas M, Fasano RM, Field JJ, Hendrickson JE, Howard J, et al. American Society of Hematology 2020 guidelines for sickle cell disease: transfusion support. Blood Adv 2020; 4: 327-355. doi: 10.1182/bloodadvances.2019001143.
5. Lal A, Wong T, Keel S, Pagano M, Chung J, Kamdar A, et al. The transfusion management of beta thalassemia in the United States. Transfusion 2021; 61: 3027-3039. doi: 10.1111/trf.16640.
6. Italian National Blood Center. [National Blood self-sufficiency programme for the year 2022.] Available at: https: //www.centronazionalesangue.it/wp-content/uploads/2022/07/D.M.-26.05.2022_Programma-nazionale-autosufficienza-2022_GU-04.07.22.pdf. Accessed on 19/01/2023. [in Italian.]
7. SITE [internet].[Census of health facilities where patients with hemoglobinopathies are followed up.] Available at: https: //www.site-italia.org/centri.php?166513241660.78. Accessed on 19/01/2023 [in Italian.]
8. Repertoire Acts n. 90/CSR of 17 June 2021- concerning "Update of the State-Regions Agreement of 20 October 2015 (Repertoire Acts n. 168/CSR) regarding the unit price of sale, between Health Trusts and between Regions and Autonomous Provinces, of units of blood , its components and plasma-derived drugs produced under convention, as well as actions to encourage exchange between healthcare companies within the region and between regions. Available at: https://www.statoregioni.it/media/3737/p-6-csr-atto-rep-n-90-17 giu 2021.pdf. Accessed on 19/01/2023 [in italian.]
9. Weisbach V, Riego W, Strasser E, Zingsem J, Ringwald J, Zimmermann R, et al. The in vitro quality of washed, prestorage leucocyte-depleted red blood cell concentrates. Vox Sang 2004 Jul; 87(1): 19-26. doi: 10.1111/j.1423-0410.2004.00526.x. PMID: 15260818.
10. Padmanabhan A, Connelly-Smith L, Aqui N, Balogun RA, Klingel R, Meyer E, et al. Guidelines on the Use of Therapeutic Apheresis in Clinical Practice - Evidence-Based Approach from the Writing Committee of the American Society for Apheresis: The Eighth Special Issue. J Clin Apher; 34: 171-354. doi: 10.1002/jca.21705.
11. Mukherjee S, Sahu A, Ray GK, Maiti R, Prakash S. Comparative evaluation of efficacy and safety of automated versus manual red cell exchange in sickle cell disease: A systematic review and meta-analysis. Vox Sang 2022; 117: 989-1000. doi: 10.1111/vox.13288.
12. Davis BA, Allard S, Qureshi A, Porter JB, Pancham S, Win N, et al. Guidelines on red cell transfusion in sickle cell disease. Part I: principles and laboratory aspects. Br J Haematol 2017; 176: 179-191. doi: 10.1111/bjh.14346.
13. Ang AL, Lim CY, Ng WY, Lam JCM. Non-transfusion dependent thalassemia is independently associated with higher alloimmunization risk than transfusion dependent thalassemia and would benefit the most from extended red cell antigen-matching. Transfusion 2021; 61: 2566-2577. doi: 10.1111/trf.16590.
14. White J. Red cell antibodies – clinical significance or just noise?. ISBT Sci Ser 2017; 12: 19-24. Available at: https: //doi.org/10.1111/voxs.12325. Accessed on 19/01/2023.
15. Franchini M, Forni GL, Marano G, Cruciani M, Mengoli C, Pinto V, et al. Red blood cell alloimmunisation in transfusion-dependent thalassaemia: a systematic review. Blood Transfus 2019; 17: 4-15. doi: 10.2450/2019.0229-18.
16. Shastry S, Chenna D, Basavarajegowda A, Das S, Chaudhary RK. Red blood cell alloimmunization among recipients of blood transfusion in India: A systematic review and meta-analysis. Vox Sang 2022; 117: 1057-1069. doi: 10.1111/vox.13296.
17. Graziadei G, De Franceschi L, Sainati L, Venturelli D, Masera N, Bonomo P, et al. Transfusional approach in multi-ethnic sickle cell patients: real-world practice data from a multicenter survey in Italy. Front Med (Lausanne) 2022; 9: 832154. doi: 10.3389/fmed.2022.832154.

18. Milkins C, Berryman J, Cantwell C, Elliott C, Haggas R, Jones J, et al. Guidelines for pre-transfusion compatibility procedures in blood transfusion laboratories. British Committee for Standards in Haematology. Transfus Med 2013; 23: 3-35. doi: 10.1111/j.1365-3148.2012.01199.x. Erratum in: Transfus Med 2022; 32: 91. PMID: 23216974.
19. Smart E, Armstrong B, Lee E. Blood group systems. ISBT Sci Ser 2020; 15 (S1): 123-150. Available at: https: //doi.org/10.1111/voxs.12593. Accessed on 19/01/2023.
20. Reid ME, Lomas-Francis C, Olsson ML. *The blood group antigen: facts book*. 3rd edition. Amsterdam: Academic Press, Elsevier; 2013.
21. Sunassee A, Mackey J, Anderson KA, Huntington MK. Acute hemolytic transfusion reaction due to anti-Kpa antibody. S D Med 2018; 71: 222-223. PMID: 29999609.
22. Koshy R, Patel B, Harrison JS. Anti-Kpa-induced severe delayed hemolytic transfusion reaction. Immunohematology 2009; 25: 44-47. PMID: 19927619.
23. Klein HG, Anstee DJ. *Mollison's blood transfusion in clinical medicine.* 12th Edition. London: 2014 Wiley-Blackwell; p. 214-218.
24. Coleman S, Westhoff CM, Friedman DF, Chou ST. Alloimmunization in patients with sickle cell disease and underrecognition of accompanying delayed hemolytic transfusion reactions. Transfusion 2019; 59: 2282-2291. doi: 10.1111/trf.15328.
25. Gupta R, Singh DK, Singh B, Rusia U. Alloimmunization to red cells in thalassemics: emerging problem and future strategies. Transfus Apher Sci 2011; 45: 167-170. doi: 10.1016/j.transci.2011.07.014.
26. Vichinsky E, Neumayr L, Trimble S, Giardina PJ, Cohen AR, Coates T, et al. CDC Thalassemia Investigators. Transfusion complications in thalassemia patients: a report from the Centers for Disease Control and Prevention (CME). Transfusion 2014; 54: 972-81; quiz 971. doi: 10.1111/trf.12348.
27. Zalpuri S, Zwaginga JJ, le Cessie S, Elshuis J, Schonewille H, van der Bom JG. Red-blood-cell alloimmunization and number of red-blood-cell transfusions. Vox Sang 2012; 102: 144-149. doi: 10.1111/j.1423-0410.2011.01517.x.
28. Ang AL, Lim CY, Ng WY, Lam JCM. Non-transfusion dependent thalassemia is independently associated with higher alloimmunization risk than transfusion dependent thalassemia and would benefit the most from extended red cell antigen-matching. Transfusion 2021; 61: 2566-2577. doi: 10.1111/trf.16590.
29. Chou ST, Jackson T, Vege S, Smith-Whitley K, Friedman DF, Westhoff CM. High prevalence of red blood cell alloimmunization in sickle cell disease despite transfusion from Rh-matched minority donors. Blood 2013; 122: 1062-1071. doi: 10.1182/blood-2013-03-490623.
30. Gerritsma J, Bongaerts V, Eckhardt C, Heijboer H, Nur E, Biemond B, van der Schoot E, et al. Extended phenotyping does not preclude the occurrence of delayed haemolytic transfusion reactions in sickle cell disease. Br J Haematol 2022; 196: 769-776. doi: 10.1111/bjh.17875.
31. Kacker S, Ness PM, Savage WJ, Frick KD, Shirey RS, King KE, et al. Cost-effectiveness of prospective red blood cell antigen matching to prevent alloimmunization among sickle cell patients. Transfusion 2014; 54: 86-97. doi: 10.1111/trf.12250.
32. Luken JS, Ritsema SP, Van der Wal MM, van der Schoot CE, Rouwette EAJA, de Haas M, et al.: Mapping anticipated advantages and disadvantages of implementation of extensive donor genotyping: A focus group approach. Transfus Med 2022; 32: 366-374. doi: 10.1111/tme.12887.
33. Decree of the President of the Council of Ministers of 3 March 2017. Identification of surveillance systems and registers of mortality, cancer and other pathologies. Official Gazette General Series n.109 of 12-05-2017. Available at: https: //www.gazzettaufficiale.it/eli/id/2017/05/12/17A03142/sg. Accessed on 19/01/2023 [in Italian.]
34. National Registry for Thalassemia and Hemoglobinopathies. Available at: https: //www.centronazionalesangue.it/registro-nazionale-talassemie-ed-emoglobinopatie/. Accessed on 19/01/2023 [in Italian.]
35. Longo F, Corrieri P, Origa R, Barella S, Sanna PMG, Bitti PP, et al. Changing patterns of thalassaemia in Italy: a WebThal perspective. Blood Transfus 2021; 19: 261-268. doi: 10.2450/2020.0143-20.
36. Sicilian Region. Department of Health - Department of Health Activities and Epidemiological Observatory, Service 9 "Surveillance and evaluation epidemiology": Report of the Sicilian Thalassemia and Hemoglobinopathies Registry (RESTE) - Update to 31/12/2021. Available at: https: //www.regione.sicilia.it/sites/default/files/2022-07/relazione%20registro%20talassemia%20al%2031-12-2021%20%281%29.pdf. Accessed on 19/01/2023 [in Italian.]
37. Noori T, Ghazisaeedi M, Aliabad GM, Mehdipour Y, Mehraeen E, Conte R, et al. International comparison of Thalassemia Registries: challenges and opportunities. Acta Inform Med 2019; 27: 58-63. doi: 10.5455/aim.2019.27.58-63.
38. Italian Society Thalassemia and Hemoglobinopathies [SITE]. Architecture of the Italian Thalassemia and Hemoglobinopathies Network, second version: January 2022. Available at: https://www.site-italia.org/protocolli.php?168137100503. Accessed on 19/01/2023. [in Italian.]
39. Trompeter S, Massey E, Robinson S; Transfusion Task Force of the British Society of Haematology Guidelines Committee. Position paper on International Collaboration for Transfusion Medicine (ICTM) Guideline 'Red blood cell specifications for patients with hemoglobinopathies: a systematic review and guideline'. Br J Haematol 2020; 189: 424-427. doi: 10.1111/bjh.16405.
40. Tormey CA, Hendrickson JE. Transfusion-related red blood cell alloantibodies: induction and consequences. Blood 2019; 133: 1821-1830. doi: 10.1182/blood-2018-08-833962.
41. Williams LA 3rd, Lorenz RG, Tahir A, Pham HP, Marques MB. High percentage of evanescent red cell antibodies in patients with sickle cell disease highlights need for a national antibody database. South Med J 2016; 109: 588-591. doi: 10.14423/SMJ.0000000000000528.
42. Mathur G, Wilkinson MB, Island ER, Menitove JE, Tilzer L. A case for a national registry of red blood cell antibodies. Vox Sang 2022; 117: 738-740. doi: 10.1111/vox.13250.
43. Powell Z, Jiang N, Shrestha R, Jackson DE. Would a National Antibody Register contribute to improving patient outcomes? Blood Transfus 2022; 20: 132-142. doi: 10.2451/2021.0421-20.
44. Angastiniotis M, Cannon L, Antoniou E, Brunetta AL, Constantinou G, Knoll EM, et al. Hemoglobin disorders in Europe: a systematic effort of identifying and addressing unmet needs and challenges by the Thalassemia International Federation. Thalassemia Reports, 11 (1). Available at: https: //doi.org/10.4081/thal.2021.9803. Accessed on 19/01/2023.
45. Oviedo P, Jaffré Y, Danic B, Mazières S, Chiaroni J. Impact of societal and legal context on the blood supply of African-ancestry populations in Western countries: A review of practices and the French example. Vox Sang 2022; 117: 1137-1144. doi: 10.1111/vox.13326.
46. General Directorate of Digitization. Health Information System and Statistics, Statistics Office: Certificate of assistance to childbirth (CeDAP), Analysis of the birth event-Year 2021, Available at: C_17_pubblicazioni_3264_allegato.pdf (salute.gov.it), p. 23-26. Accessed on 19/01/2023 [in italian.]

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