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## RESEARCH ARTICLE

### ABO GROUP DISTRIBUTION: A RECENT DRIFT IN THE "ITALIAN" RESIDENT WOMEN IN MILAN AREA

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#### ABSTRACT

**Background and Objectives:** This study was aimed to identify the distribution patterns of ABO and Rh-D blood group among the women hospitalized in our hospital Pio X, during the years 2009-2018 in order to observe the new distribution in the Milan population. **Materials and Methods:** We collected and analyzed 13223 blood samples and evaluate the distribution of ABO and Rh (D) antigens also by ethnic groups. **Results:** We observed that during last ten years the resident population is changed; the percentage of Caucasian (alias Italian) women is reduced, while the percentage of women belonging to other ethnic groups is increased. This different composition of resident population reflected what we observed in ABO and Rh distribution. We noted that the actual Milan population is formed by women of about 10 % of African, 8 % of East Asian, 8 % of Iberia-American, 5 % of east Europe, and around 70 % of Caucasian. We observed this difference in a new distribution of ABO group and Rh antigens. **Conclusion:** Our data suggest that the Milan population is composed by Caucasian people but also by mixed marriages between Caucasian and East Asian population children, in particular. This fact could lead an important remix of blood genes that explained the different distribution also in the actual Milan resident population.

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#### INTRODUCTION

ABO groups have been discovered in 1901 by Karl Landsteiner and they have represented a great instrument for the study of hereditarity and for the clinical interest (Dean, 2012 and Watkins, 2011). Since 1901, more than 20 distinct blood group systems have been identified but the ABO and Rhesus blood groups remain clinically the most important. The gene that determines human ABO blood type is located on chromosome 9 (9q34.1) and is called ABO glycosyltransferase. The ABO locus has three main allelic forms: A, B, and O, as mentioned above and each of them is responsible for the production of its glycoprotein. The allele A and B (IA and IB) are dominant over the allele O (i) and they expressing a special dominance relationship (codominance), which means that type A and type B parents can have an AB-type child and O-type child if they are both heterozygous (IBi, IAi). It is therefore the combination of alleles inherited by parents that determines which glycoproteins (or antigens) are found on persons' blood cells and, thereby, their ABO blood type (Yazer, 2006).

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Furthermore, they are also well defined genetic markers employed in population genetics (Reid, 2002; Reid, 1995 and Sigmon, 1992), and exhibit polymorphism in different populations. A total of 30 human blood group systems are now recognized by the International Society of Blood Transfusion. ABO blood groups are the most investigated blood group system, and owing to ease of identifying their phenotypes, they have been used as genetic markers of populations. It is well established that differences in ABO blood groups exist, both within and among ethnic groups and by geographical areas. Some variations may even occur in different areas within one small country. The distribution of ABO and RhD blood groups varies throughout the world. In Italy there is only one recent work in which the distribution among the groups has been studied (Villa, 1996). The other studies have been performed by AVIS (Associazione Volontari Italiani del Sangue) and the group percentage for group O, that is the most frequent, is 46%, for A is 42%, for B is 9% and for AB 3%. Both blood groups both their antigens have been used to study the incidence and causality of several diseases, such as cancer, diabetes, infection diseases and heart pathologies (Farhud, 2013; Hamed, 2012 and Yazer, 2016), or for their protective

role against several illness or infection (e.g malaria) (Behal, 2010; Cooling, 2015 and Kuesap, 2018). In the gynaecology department, they are useful to identify possible haemolytic pathologies occurring in new-borns, usually related to Rh antigens (Birchenall, 2013). In this study we analyzed the ABO group and Rh antigens distribution in all the women hospitalized in our institute between 2009 and 2018 in order to determinate which is the new distribution of Italian ABO group and Rh antigens.

## MATERIALS AND METHODS

We have done a retrospective study from 1<sup>st</sup> January of 2009 to 31<sup>st</sup> October of 2018 on a selected population of Pio X Hospital of Milan. We have studied only mothers that arrived in our hospital during pregnancy and we have collected 13223 blood groups. All antibody screening and compatibility testing using Ortho BioVue System (Ortho-Clinical Diagnostics GmbH) cassettes technology and digital picture editing. This system provides automated test editing functions such as liquid pipetting, cassette handling, incubation, centrifugation, reaction grading and interpretation as well as data management. The AutoVue blood bank automation may be used as an independent system as well as connected to the customer laboratory information system (LIS) in terms of a bidirectional link with sample data passing from the LIS to the AutoVue and test results passing from the AutoVue to the LIS. The principle of measurement and grading is based on a camera with coupling charge device, which reads both front and back side of BioVue cassettes 25 times. Reaction interpretation is done using a 342 Transfus image processing system (IPS) software and a grading algorithm.

The original picture of each cassette is saved in a JPG format on a hard. The test principle of the Ortho BioVue System is the column agglutination technique using columns containing reagents and glass spheres. By centrifugation of the cassettes agglutinated erythrocytes form a layer on top of the reagent-ball mix, whereas non-agglutinated erythrocytes sink to the bottom of the column. Ortho BioVue System cassettes consist of 6 columns which contain reagents in a buffered solution. One of the most important innovations of the AutoVue is the possibility to start emergency analysis during routine diagnostics. Using this random access modus, blood typing with antibody screening test and 6 cross-matches needs 25 min; if the blood group is known, this analysis time can be reduced to 22 min. All results are divided for ethnicity origin by our front office (Isolabella Dedalus) in order with the data collected at the time of acceptance of the blood sample, defined by born place, and we analyzed again all results based on the age of mothers divided in three groups: 18-35 yrs., 26-35 yrs. and 36-45 yrs.

## RESULTS

We evaluate blood group distribution in women admitted in our hospital during ten years from 2009 to 2018. First of all, we observed that the blood group distribution changes compared to what we observed in literature (AVIS). In fact, in literature is reported that O was the most frequent (46%), followed by A (42%), B (9%) and AB (3%) but we observed some differences in ABO distribution. In fact, although O group is the most frequent, we observed a different percentage. In the last 10 years in the hospital Pio X in Milan, we observed 43.5 % of women with O group, 34.8% with A group, 15.8% with B and 6% with AB (Table 1).

Table 1. Percentage of ABO distribution in literature and in our hospital during the years 2009-2018

	O	A	AB	B
AVIS	46.0	42.0	3.0	9.0
Pio X	43.5	34.8	6.0	15.8

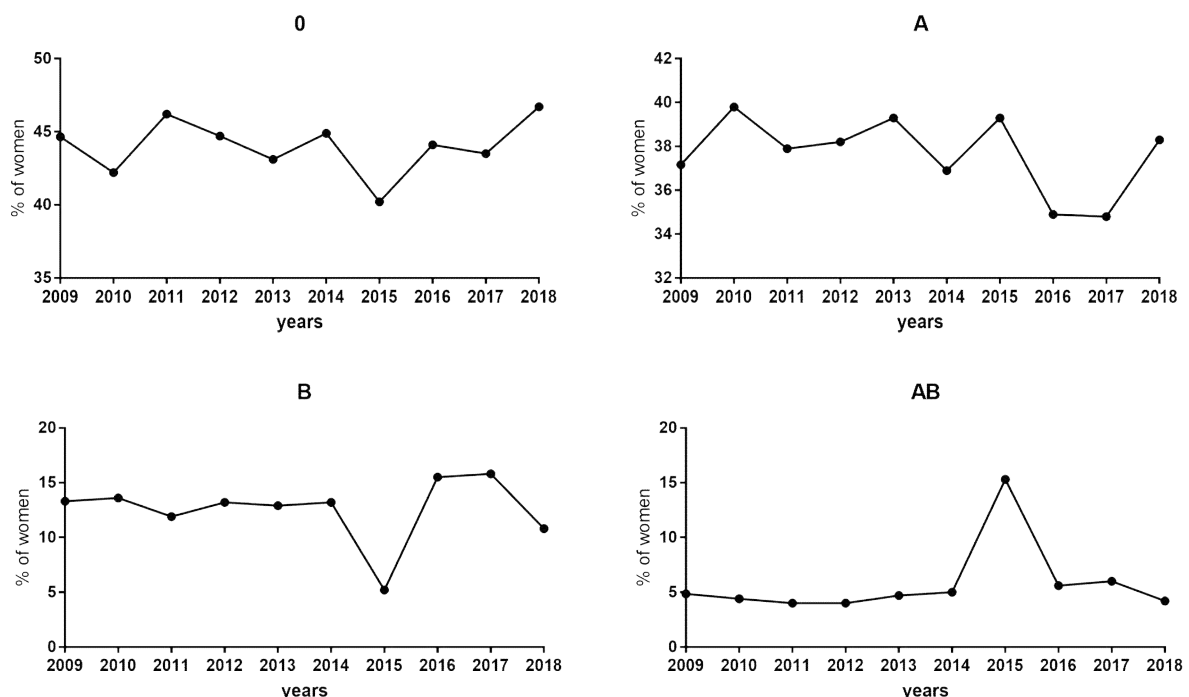


Figure 1. Trends in ABO group distribution from 2009 to 2018

This trend is not constant during these years; for example we noticed a reduction of O and B groups of women in 2015, and dramatic increase in women with AB group (Fig 1), and antigens that define Rh phenotype present some differences compared to what we observed in literature (Table 2 and Fig 2). In fact, despite the general distributions are very similar, there are some variation in term of percentage; in particular we observed a very significant trend with two flexion points for the year 2015 and 2017 (Fig 2).

These data prompted us to investigate whether this different distribution of ABO groups and Rh phenotype was due to different ethnicity of hospitalized women. The majority of the hospitalized women of our hospital belong to Caucasian group (70-85% EU, Fig 3 A), while the women belonging the other ethnic groups are circa 5-10 % for each group (Fig 3A). We observed that, during the past three years (2015-2018) the Caucasian access are reduced in favor of the increase of the other ethnic groups (Fig 3 B).

**Table 2. Percentage of Rh antigen distribution in literature and in our hospital during the years 2009-2018**

	0 -	0 +	A -	A +	AB -	AB +	B -	B +
<b>AVIS</b>	7.0	39.0	6.0	36.0	0.5	2.5	1.5	7.5
<b>Pio X</b>	4.8	41.9	3.6	34.7	0.6	3.6	1.8	9.0

**Table 3. Number of hospitalized women in the years 2009-2010 divided by age and ethnicity**

		2009-2010 yrs							
		0-	0+	A-	A+	AB-	AB+	B-	B+
<b>18-25 yrs</b>	<b>A</b>	1	15	1	12	0	2	0	3
	<b>EA</b>	1	3	0	3	0	1	0	4
	<b>EU</b>	16	81	16	58	1	11	4	26
	<b>IA</b>	0	4	0	2	0	0	0	2
	<b>R</b>	1	7	1	7	0	3	1	3
<b>26-35 yrs</b>	<b>A</b>	3	22	1	17	2	10	0	7
	<b>EA</b>	3	20	1	19	1	4	1	23
	<b>EU</b>	87	580	88	568	9	61	34	205
	<b>IA</b>	1	42	3	16	0	1	0	6
	<b>R</b>	7	24	4	20	0	7	3	11
<b>36-45 yrs</b>	<b>A</b>	2	21	3	11	0	0	0	6
	<b>EA</b>	1	8	2	9	0	1	0	9
	<b>EU</b>	107	663	70	613	6	66	37	155
	<b>IA</b>	2	33	2	10	0	1	0	4
	<b>R</b>	4	8	1	5	1	1	0	3

**Table 4. Number of hospitalized women in the years 2015-2016 divided by age and ethnicity**

		2015-2016 yrs							
		0-	0+	A-	A+	AB-	AB+	B-	B+
<b>18-25 yrs</b>	<b>A</b>	2	2	0	5	0	2	1	3
	<b>EA</b>	0	7	1	3	0	0	0	7
	<b>EU</b>	5	25	4	28	0	1	2	10
	<b>IA</b>	0	1	1	2	0	0	0	2
	<b>R</b>	1	5	0	4	0	0	0	2
<b>26-35 yrs</b>	<b>A</b>	1	20	1	7	0	3	0	4
	<b>EA</b>	0	26	1	12	0	2	0	8
	<b>EU</b>	31	220	22	209	3	35	14	90
	<b>IA</b>	1	12	2	6	0	2	0	8
	<b>R</b>	2	10	1	23	0	2	2	1
<b>36-45 yrs</b>	<b>A</b>	1	4	0	8	0	0	1	0
	<b>EA</b>	0	4	0	6	0	2	1	5
	<b>EU</b>	27	190	29	201	3	25	7	60
	<b>IA</b>	1	17	1	5	0	0	0	1
	<b>R</b>	6	6	1	5	0	1	3	3

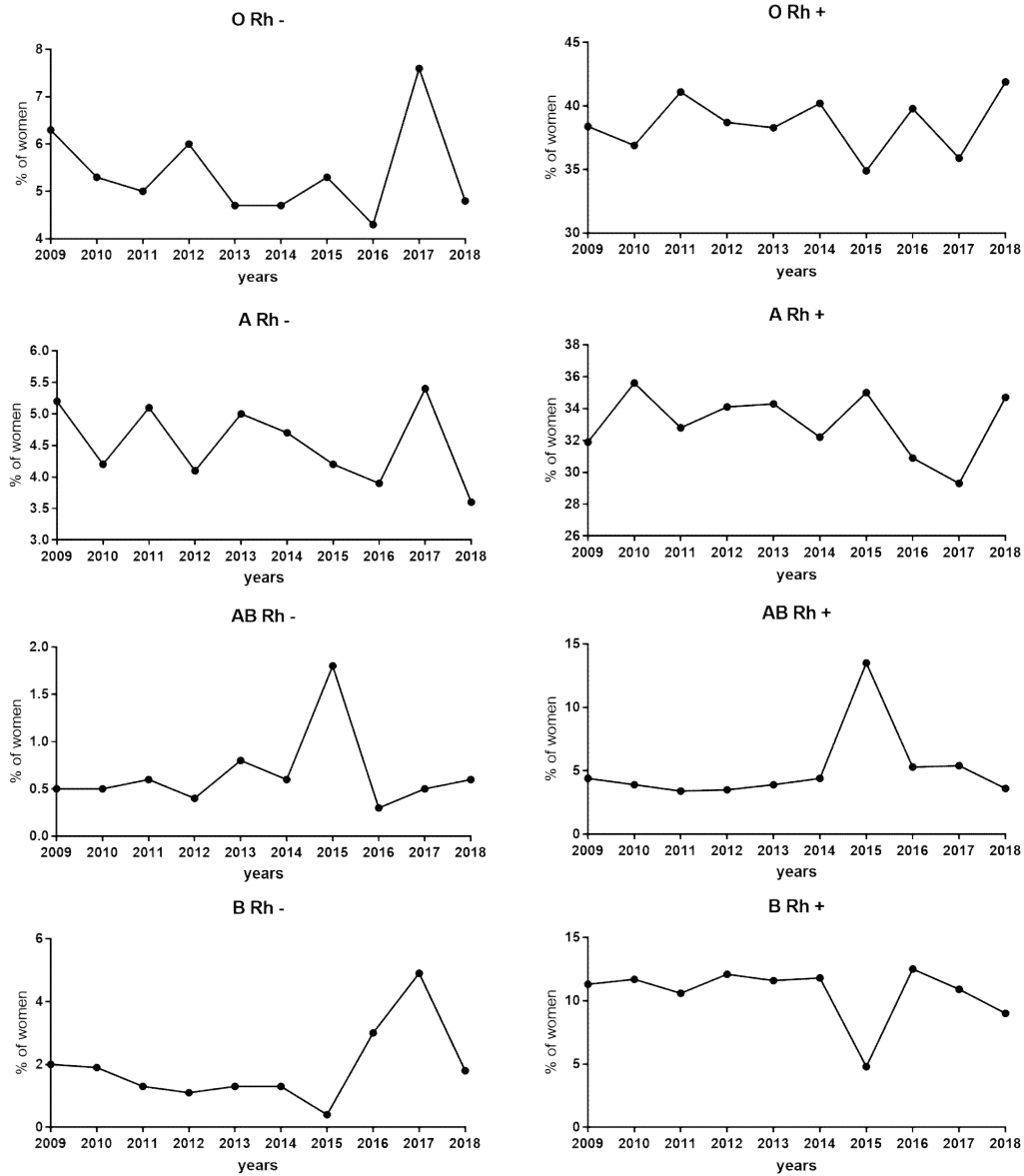


Figure 2. Trends in Rh antigen distribution from 2009 to 2018

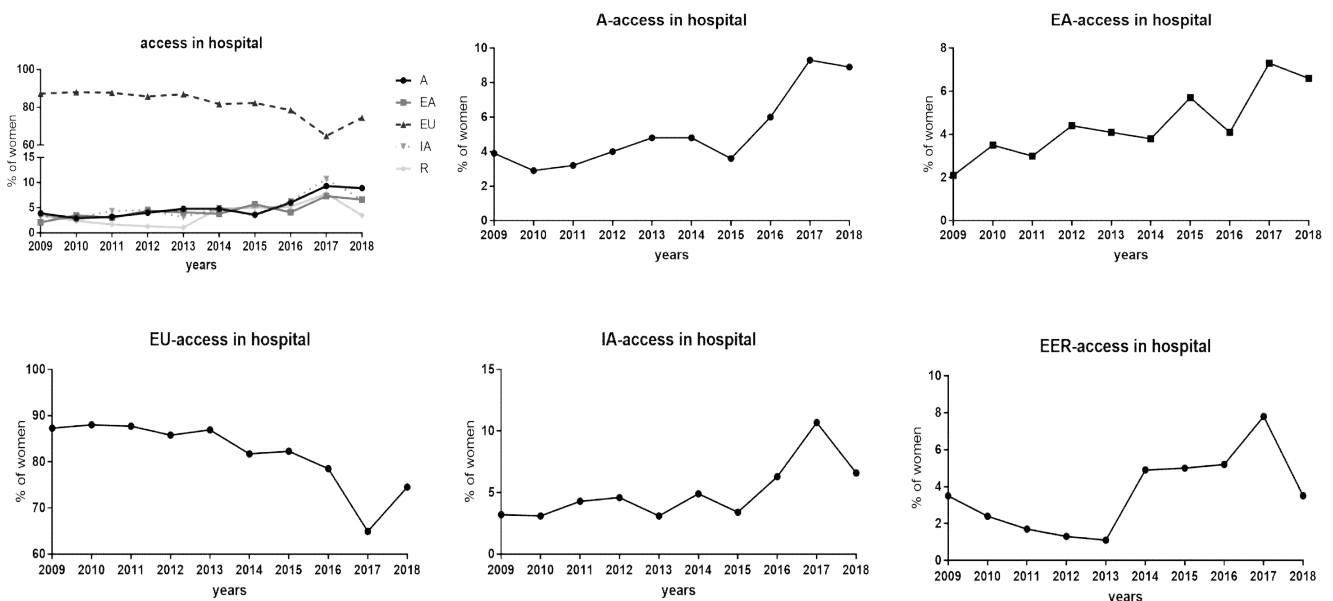


Figure 3. Ethnic group hospitalization. A) Graph represents the percentage of all hospital access divided for ethnic group in the years 2009-2018; B) graphs represent the hospital access during 2009-2018 for each ethnic groups. Letters represent the different group: A=African; EA=East Asian; EU=Caucasian; IA=Ibero-American; R=Russian-East Europe

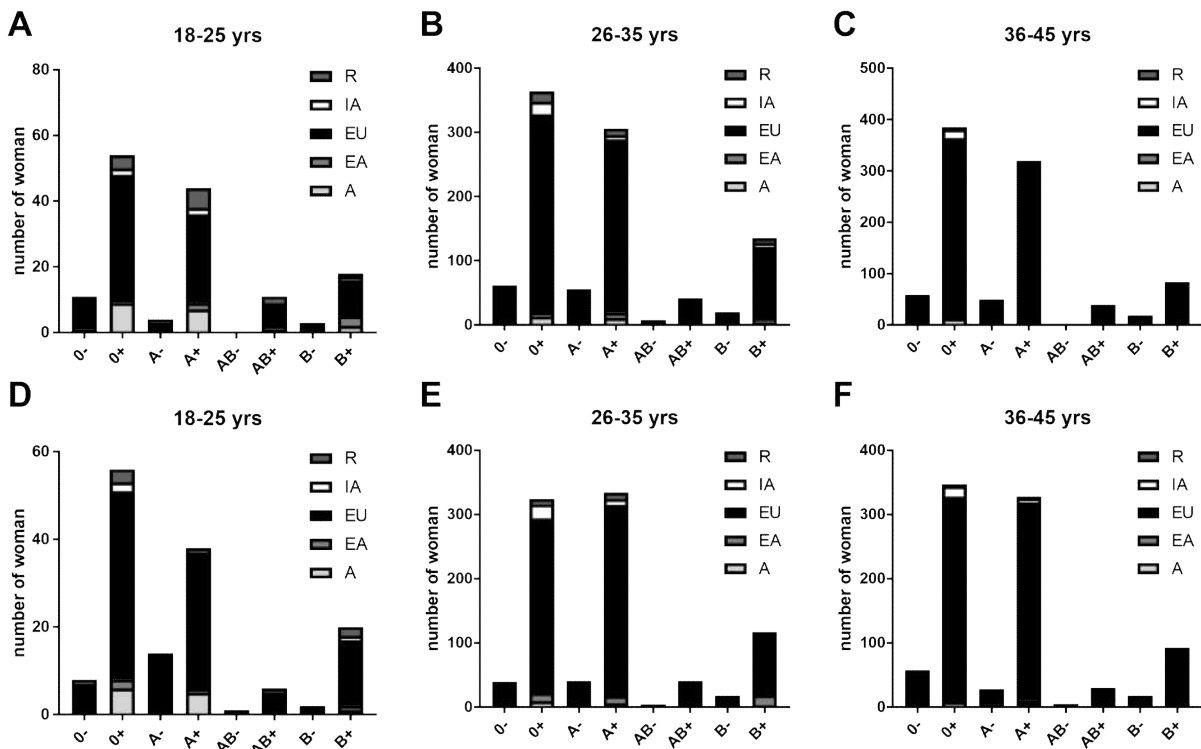


Figure 4. Number of women for each ethnic groups in the years 2009 (A-C) and 2010 (D-F) divided for age groups. Letters represent the different group: A=African; EA=East Asian; EU=Caucasian; IA=Ibero-American; R=Russian-East Europe

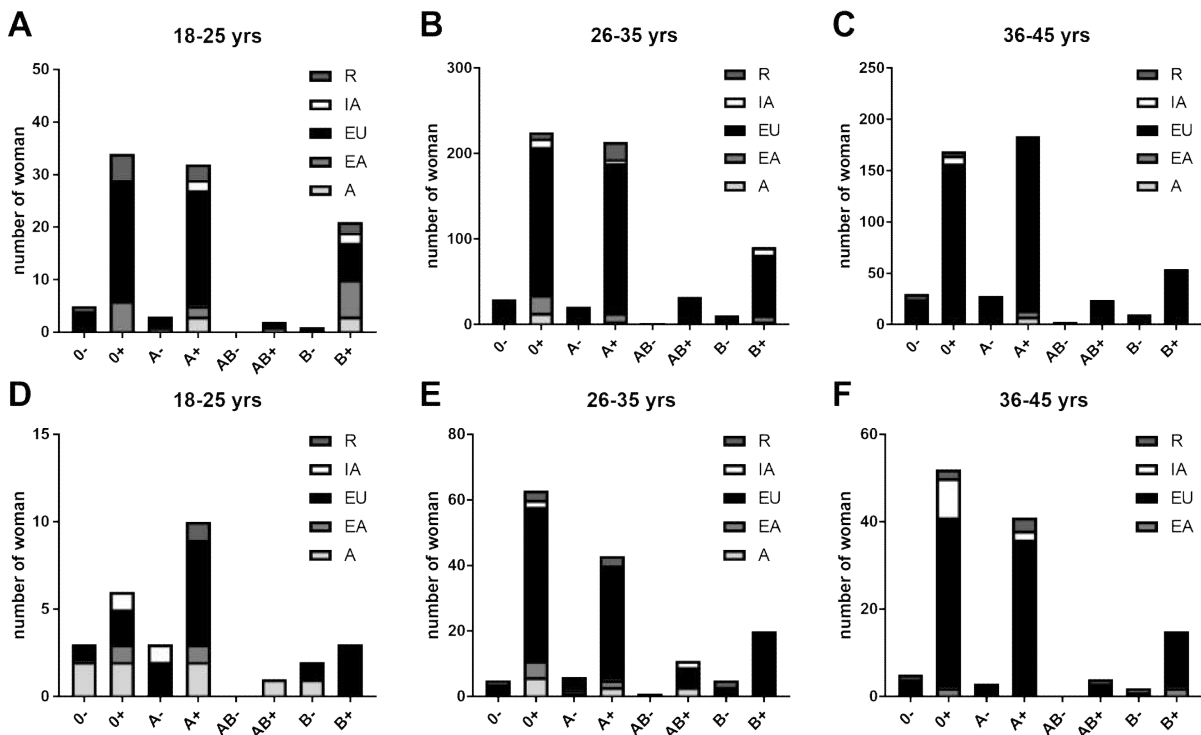


Figure 5. Number of women for each ethnic groups in the years 2015 (A-C) and 2016 (D-F) divided for age groups. Letters represent the different group: A=African; EA=East Asian; EU=Caucasian; IA=Ibero-American; R=Russian-East Europe

Moreover, we observed that the different AB0 and Rh loci distribution is a recent process and is not dependent by the age of women analyzed. In fact, during the first two years analyzed, 2009 and 2010, we did not observe any variation in term of group representation and this is also true if we classify the women by their age (Fig 4, Table 3), in contrast between 2015 and 2016 (Fig 5, Table 4) there are a very interesting shift. In fact, for the women between 18 and 25 years different pattern of ethnic distribution for the groups O Rh D and d, A Rh D and d and for the group B Rh D (Fig 5 A and B).

The group of women between 26 and 35 years old remains constant (Fig 5 C and D), while the women between 36 and 45 years old show a different pattern for the group O Rh D, A Rh D and B Rh D (Fig 5 E and F). The ethnic pattern variations are less visible in all Rh d groups, because the fewer number of women.

**DISCUSSION**

In the recent study, we analyze 13223 hospitalized women, and we analyze their ABO group and their Rh phenotype. In Italy

the last previous study of the ABO and Rh distribution dates back to 1996 [7] and their samples regard 250 healthy donors. Other data collection were performed by AVIS (Associazioni Volontari Italiani del Sangue) and belong to the year 2017. Given the importance of the correlation between ABO groups and Rh phenotype to several diseases, we evaluated the women hospitalized in Pio X Institute. We observed that in the Italian population there are a shift in term of ABO and Rh group distribution, despite the trend is similar to the previous. In fact we observed that 0 group is the most represented and it is very constant (46%), the A group was about 42 % and now is represented around 38%; the B group was about 9% and we observe in our hospital about 14 %, and at least AB was about 3 % and it is actually about 5 %. The hospital Pio X is a hospital without emergency room, and we usually admit women that are under the care of our personnel. Taken together this consideration and the previous data we speculate that the hospitalized women in our institute represent the "new resident" women of Milan. From the data collected the results link especially with the percentage of B and AB groups, are not explainable with the accesses in our hospital. In fact the percentage of the East Asian women that usually have the greater percentage of B and AB group is only around the 8%. By the study of the Milan story we found a very interesting thing about the immigration of East Asian population. In the book of Ciaj Rocchi e Matteo Demonte: "La storia di mio nonno Wu – come le storie dei pionieri – ha contribuito ad aprire la via dell'integrazione cinese nell'Europa contemporanea." [15] it is described that the immigration by East Asia it began in the early 30s in Italy and particularly in the northern cities: Turin, Brescia, Milan. These new data prompts us to suppose that the Milan population is composed by Caucasian people but also by mixed marriages between Caucasian and East Asian population children. This fact could lead an important remix of blood genes that explained the different distribution also in the actual Milan resident population. Altogether data suggest that the actual Milan/Italian population is formed by a 10 % of African origin women, 8 % of East Asian women, 8 % of Iberia-American women, 5 % of east Europe women, and around 70 % of Caucasian women and we could observe this difference in the new distribution of ABO group and Rh antigens.

**Conflicts of Interest:** The authors declare no competing financial interests.

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