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Cytomegalovirus in Blood Donors: IgG Detection by ELISA Technique in Analamanga Transfusion Center, Antananarivo

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Abstract:

- ***Introduction:***

Blood transfusion is one of the routes of transmission of cytomegalovirus (CMV) infection, which puts immunocompromised subjects at risk. To ensure the blood transfusion safety, this study aims to determine the CMV seroprevalence in blood donors seen at the CRTS Analamanga.

- ***Method:***

A 3-month prospective descriptive and analytical study was conducted from June to August 2021. Medically selected, consenting candidates were included. Anti-CMV IgG antibodies were screened by ELISA using the Fortress Diagnostics® CMV IgG kit. IgG avidity detection was not performed. HIV, hepatitis B and C and syphilis infections were assessed in parallel.

- ***Results:***

A further 2,131 donors were included in the present study. Mean age was 33.7 years, with M/F sex ratio of 2.7. Family-replacement donors were in the predominant proportion (85.4%). CMV infection prevalence was 92.4%, mainly in the 20-29 (92.7%) and 30-39 (93.9%) age groups, with statistical significance correlating with age (p=0.17). CMV and hepatitis B virus coinfections were detected in 73 donors (3.7%).

- ***Conclusion:***

IgG anti-CMV antibodies remain high in Malagasy blood donors. These findings suggest the need to introduce its systematic screening or leukoreduction procedures for blood products in Malagasy transfusion centers.

Keywords: *Cytomegalovirus - Blood Donors- ELISA – Prevalence.*

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I. INTRODUCTION

➢ *Background*

To promote effective blood transfusion safety, good practice includes infectious safety. Screening all blood donors for blood-borne infections is mandatory, particularly viral infections. In Madagascar, detection concerns hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV) and syphilis.

Despite scientific advances in screening for infectious agents, the risk of transmission through blood components remains [1]. In Western countries, risk has been considerably reduced by a policy of increasing the voluntary donor base, rigorous medical screening and molecular biology techniques for infectious agents. Screening cytomegalovirus (CMV) blood donors is an integral part of the biological validation of blood components. In fact, although the majority of infected adults are healthy carriers, CMV plays an important role in post-transfusion syndromes especially in newborns and immunocompromised patients such as patients with cancer and transplant patients[2-3]. Prevalence in the worldwide population varies from 60 to 100% [1]. Clinical and hematological CMV-related post-transfusion syndromes are relatively benign in immunocompetent subjects. They can be serious, however, in immunocompromised patients, causing encephalitis, retinitis, pneumonia.

In France, CMV infection frequency ranges from CMV to 47% in the Paris region, while in Africa it varies from 89.1% in Mali to 92.2% in Burkina Faso and even 95.8% in Nigeria among blood donors [8-11]. In industrialized countries, leukoreduction by filtration of labile blood products reduces the risk of CMV contamination [6]. In Madagascar, Ravaoarinoro et al, for an initial study carried out in 1986 on pregnant women Befelatanana Hospital found a prevalence of 80% using the complement fixation technique to identify anti-CMV antibodies [7]. In blood donors, few data are available. We aimed to assess CMV infection prevalence by screening anti-CMV IgG antibodies in blood donors at the Analamanga Regional Blood Transfusion Centre , to determine links with donors' social parameters, in order to suggest ways of making transfusion safer.

II. METHODS

A prospective, descriptive and analytical 3-month study was conducted at the Analamanga blood center from June to August 2021 including medically selected donors who had given written consent after verbal explanation . Sampling was exhaustive. Following each blood donation, 3 to 5 ml of whole blood was collected in a heparinized tube. Samples were centrifuged at 3,000 rpm for 10 minutes; plasma was used for serology of other blood-borne infections and anti-CMV Ig G antibodies. ELISA (Enzyme Linked Immunosorbent Assay) technique using Fortress Diagnostics® kit was used to screen Ig G antibodies. Using a spectrophotometer, IgG values ≥16.5 IU/mL were considered positive, while levels <13.5 IU/mL were classified as negative. Levels between 13.5 and 16.5 IU/mL were considered indeterminate. IgG avidity testing was not performed for positive samples.

Age, gender, type of donation, donor profession, ABO - Rhesus D phenotype and serological results for HBV, HCV, HIV and syphilis were also collected. Epinfo 7.0 was used to enter and process data. Pearson's Chi2 test with corresponding p-value was used to compare proportions while Fischer's exact test was adopted for comparisons where the number of people in the tables was less than 5. Statistical significance was set at p-value <0.05.

III. RESULTS

Overall, 2,131 individuals were included in the study with1,963 seen at the Analamanga Center (enter (92%) and 168 seen at mobile sites (8%). Male predominance was observed, with a male/female sex ratio of 2.7. Mean age (±SD) was 33.7 (±11.0) years, with extremes ranging from 18 to 65 years. The socio- demographic characteristics of the study population are described in **Table I.**

Table 1 Blood Donors Characteristics

Parameters	Frequency N= 2 131	Percentage (%)
Collection sites		
Fixed	1 963	92,00
Mobile	168	8,00
Profession		
Known	1 741	81,70
Unknown	390	18,30
Gender		
Male	1 550	72,70
Female	581	27,30
Age ranges (years)		
< 20	89	4,18
20-29	854	40,08
30-39	556	26,09
40-49	397	18,63
50-59	214	10,04
≥60	21	0,99

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Types of donations		
Remplacement	1 820	85,41
Regular donors	211	9,90
New volunteer	100	4,69

Anti-CMV IgG antibodies were positive in 1,969/2131 donors tested, with a prevalence of 92.4%. The test was negative in 162 cases (7.6%). Prevalence was lowest in donors under 20 years of age (83.20%), compared with 100% in those over 60 years of age. CMV infection prevalence was statistically correlated with age (p= 0.0147). Both men and women showed a high seroprevalence (p=0.384). CMV infection was most common in family replacement donors, with 1,738/1,820 positive cases (95.50%). Type of donation was strongly associated with the increased prevalence of CMV infection (p<10-5). Phenotypes A (94.15%) and O (92.68%) were most infected with CMV, but no significant difference was found **(Table II).**

Table 2 IgG anti- CMV Screening Results

Parameters	**Ig G anti-CMV positive**		**IgG anti-CMV negative**		**p-value**
	Frequency	%	Frequency	%	
Frequency	1 969	92,40	162	7,60	
Gender					
Male	1 430	92,3	120	7,70	0,384
Female	539	92,8	42	7,20	
Age ranges (years)					
< 20	74	83,2	15	16,8	**0,017**
20-29	792	92,7	62	7,3	
30-39	522	93,9	34	6,1	
40-49	367	92,4	30	7,6	
50-59	193	90,2	21	9,8	
≥60	21	100	0	0	
Types of donations					
Remplacement	1 738	95,50	82	4,50	**0,00001**
Regular	168	54,40	43	13,90	
New volunteer	63	20,2	37	11,90	
ABO phenotypes					
A	451	94,15	28	5,85	0,248
B	547	90,86	55	9,14	
AB	123	91,11	12	8,89	
O	848	92,68	67	7,32	

Serological tests for other blood-borne infections showed a higher prevalence of HBV infection (n=76/ 2,131) with 3.6%, followed by HIV infection (n=24/ 2,131) representing 1.1% of blood donors **(Figure 1**). CMV/HBV co-infection was most frequently found in 73 patients, (3.7%) of CMV-positive patients. No significant difference was found between the prevalence of CMV infection and other blood-borne infections **(Table III).**

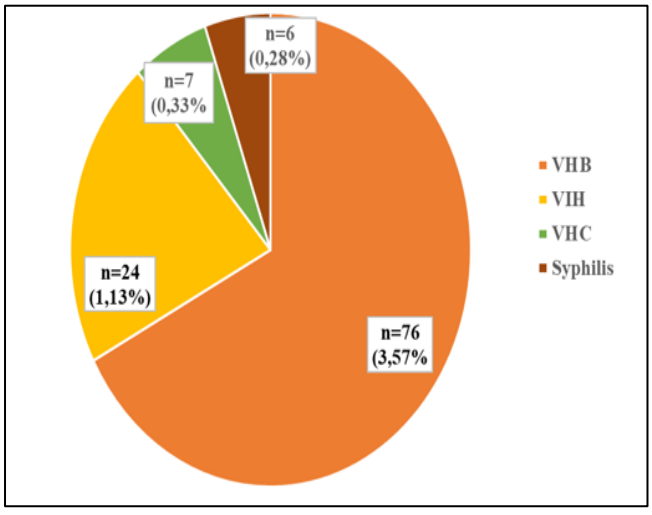

Fig 1 Frequency of Blood-Borne Bloodborne Infections other than CMV Infection

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Table 3 Association of CMV with other Transfusion Related Infections

Infections	CMV (+)			CMV (-)		Total	p-value
	Frequency n = 1 969	%		Frequency n = 162	%	N= 2 131	
Infection VIH							
Positive	22	1,10		2	1,20	24	0,555
Négative	1 947	98,90		160	98,80	2 107	
Infection VHB							
Positive	73	3,70		3	1,90	76	0,157
Négative	1 896	96,30		159	98,10	2 055	
Infection VHC							
Positive	6	0,30		1	0,70	7	0,426
Négative	1 963	99,70		161	99,30	2 124	
Syphilis							
Positive	6	0,30		0	0	6	0,622
Négative	1 963	99,70		162	100	2 125	

IV. DISCUSSION

Analamanga blood transfusion center is one of the largest in Madagascar. Over the 3-month period of study, we enrolled 2,131 donors out of a total of 2,159. Prevalence of CMV infection was high, at 92.4% (1,969 candidates selected for blood donation). IgG antibodies in these donors indicate previous contact with the virus. This high rate confirms the endemic nature of cytomegalovirus, which may be linked to socio-economic conditions that contribute to virus transmission.

Similar data are found in Africa, where the prevalence was 93.2% in 2006 in Ghana [6] and 92% among Nigerian blood donors [8]. Likewise in India, nearly 95% of blood donors are infected with CMV [16], and 97.8% in the Lahore region of Pakistan [9]. In contrast, in Western countries, the prevalence of CMV infections varies between 40% and 60% [10]. In France , the estimated prevalence of CMV infection is 50% [11]. Increases are found in populations with low socioeconomic status [12]. Prevalence is inversely proportional to the socio-economic status of the population studied [13]. Therefore, it is always a good idea to mention the profession or level of education of potential candidates for donation on blood donation screening forms.

Donors mean age was 33.7±11.0 years, with extremes of 18 and 65 years. Young adults in the 20-29 age group had a high prevalence of 92.7%. These data are similar to other African countries, such as Cameroon, with a mean age of 31.1 years [14], and Nigeria, with 32.3 years [5]. CMV seroprevalence is influenced by age: younger subjects are less exposed to the virus, but more so than older subjects [15].

A number of studies show that gender is not statistically associated with the prevalence of CMV infection [4]. Whilst a male predominance is often reported, both men and women are affected within the same age range. This male predominance is related to the high proportion of men among regular blood donors, as reported in previous studies in Madagascar [16]. The same applies to the high prevalence of CMV among family replacement donors (85.4%), who are the largest sources of blood products in Madagascar [16]. CMV prevalence among regular blood donors was 54.2%. This suggests a prior absence of screening, which could be programmed for these regular donors. Phenotypes A (94.15%) and O (93.68%) were the most CMV-infected. The phenotypic distribution of ABO erythrocyte antigens in the Malagasy population shows the predominance of the O phenotype among blood donors [17].

In Madagascar, although CMV serology is not included in laboratory tests used for biological validation of blood donations, this study resulted in CMV-negative donors who were positive for HIV (2 cases), HBV (3 cases) and HCV (1 case), which led to bag rejection. In Western countries, CMV-negative bags are required for transfusion of immunocompromised patients, as well as pregnant women and newborns. In Western countries, leukoreduction of blood products using validated and controlled techniques is commonplace [18-19].

The American Blood Bank Association has recommended transfusion of CMV-negative or leukodepleted products for subjects at risk. Such recommendations are contributing to a drastic reduction in CMV transfusion in immunocompromised patients in the USA [20]. In fact, leukoreduction reduced the risk of CMV transmission by blood products by 92.3%. Other advantages of leukoreduction include reduced alloimmunization against Human Leukocyte Antigen (HLA) antigens, prevention of non-hemolytic febrile reactions and transmission of other intraleukocytic viruses [1,21]. It is common practice for organ transplantation or transfusion in immunocompromised patients to prefer CMV-negative packed red blood cells units. Since the proportion of CMV-negative donors in the present study was low (7.60%), CMV-negative donors need to be identified and retained as blood donors for at risk subjects. They will then be made aware of the importance of maintaining their seronegative status . Implementation of leukoreduction techniques to ensure that bags have a residual leukocyte content less than 106/ml would be beneficial for the Analamanga Transfusion Center. It would greatly reduce the transmission of strict intra-leukocyte viruses, such as CMV, human T-lymphotropic virus, Epstein Barr Virus (EBV), Human Herpes Virus 6 (HHV6) and Human Herpes Virus 8 (HHV8).

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V. CONCLUSION

High rate of CMV infection (92.4%) among blood donors at the CRTS Analamanga raises the question of possible risks for the polytransfused, immunocompromised and newborns patients served by this center. Seeking for CMV-negative blood products seems difficult. Molecular Biology tools and leukoreduction methods can be used to ensure transfusion safety for those patients against blood-borne infections.

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